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Installation Restoration Research Program

Analysis of Explosives in Plant Tissues: Modifications to Method 8330 for Soil

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Prepared for Headquarters, U.S. Army Corps of Engineers

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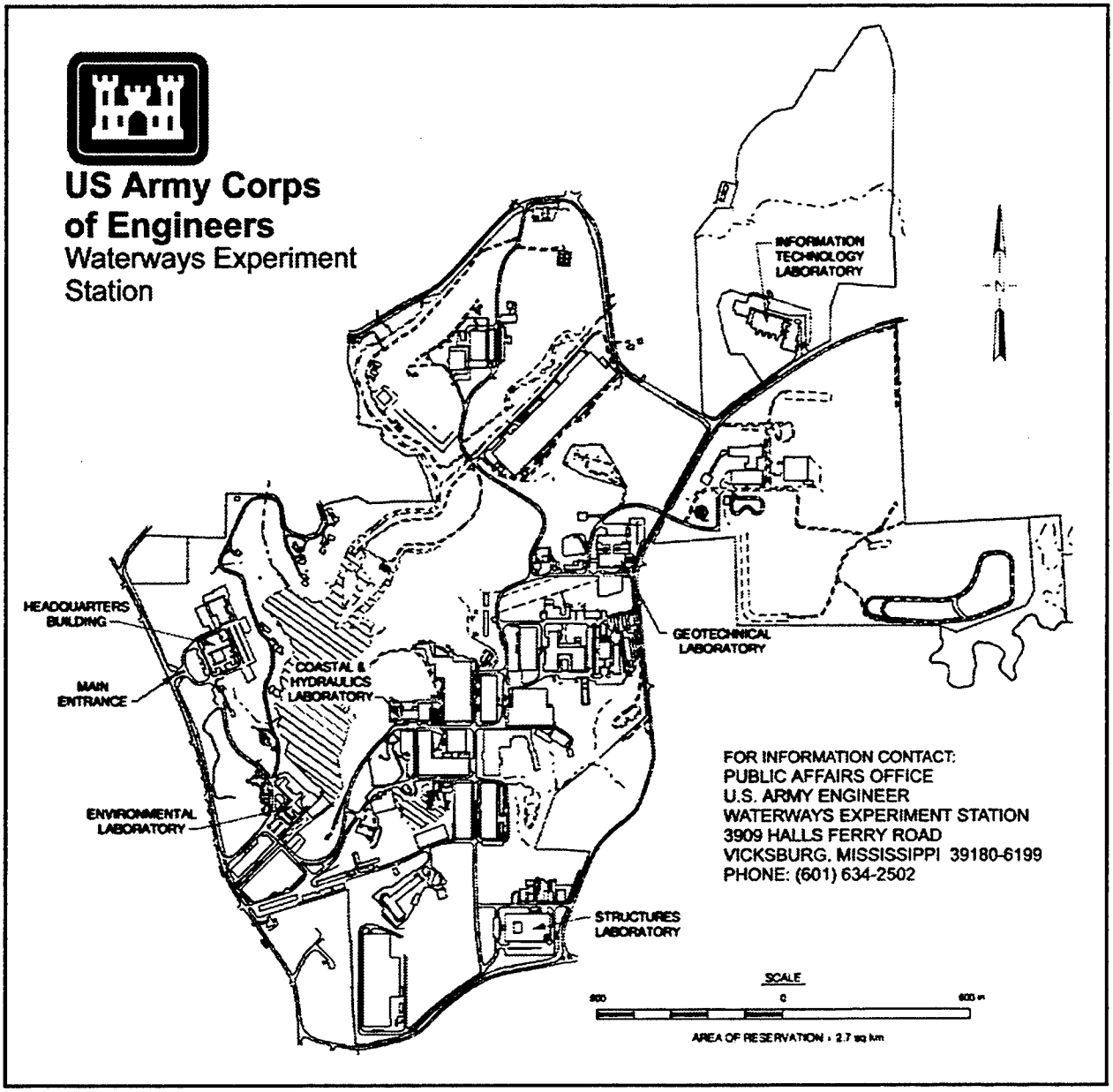

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Preface

The work reported herein was conducted by the Environmental Laboratory (EL), U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, as part of the Installation Restoration Research Program (IRRP) and the U.S. Army Environmental Quality Technology Research Program, Work Unit AF25-ET-001.

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This report was prepared by Dr. Steven L. Larson, Ms. Ann B. Strong, and Ms. Sally L. Yost, Environmental Chemistry Branch, Environmental Engineering Division (EED), EL, WES, and Ms. Lynn Escalon and Mr. Don Parker, AScI Corporation, McLean, VA. The authors gratefully acknowledge Dr. Charles Weiss and Mr. Charles White for their technical review of the report. In addition, the authors wish to acknowledge Dr. Richard A. Price and Drs. Susan Sprecher and Judy Pennington, EL, Dr. Victor Medina, University of Washington, and Dr. Elly Best, AScI, for providing plant material exposed to explosives contamination for analyses and Ms. Linda Stevenson, EL, for sample management.

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1 Introduction

Explosives and their degradation products are substances that pose a significant threat to the environment, especially in areas that have been used for synthesis of explosives, production of munitions, and use of explosives-containing ordnance. These compounds have been shown to pose a threat to the environment due to their toxicity and mutagenicity (U.S. Environmental Protection Agency (EPA) 1989, 1988a,b). The presence of explosive contaminants in soil used for agriculture and irrigation water raises questions about the levels of these contaminants in plant tissues. Analyzing of plant tissues using EPA Method 8330 for explosives in soils is inappropriate due to the large interferences and sample-handling complications encountered from the plant tissues. In order to adequately study the impact of explosives uptake by plants, a reproducible, economical, and accurate analytical method is required. This report describes a method satisfying these requirements.

The current method for determination of explosives in water and soil samples is EPA's Method 8330 (EPA 1992). While both plant tissues and soils can be considered as solid matrixes, significant differences in the two sample types require variation in the experimental techniques used for the analysis of these two matrixes. The method used for soils involves drying, weighing, extracting, sample cleanup, dilution, and analysis by reverse phase high pressure liquid chromatography (HPLC) (Jenkins 1989). The variations in the analytical process adopted in order to deal with the difference in the matrix are detailed below.

Obtaining Fresh/Wet Sample Weight

During Method 8330 for soils, the sample is weighed using an analytical balance prior to drying. This provides the analyst with a mass value that can be used to determine the percent moisture of the sample and the concentration of the explosive contaminants in the wet sample. The amount of water in soil samples is generally under 20 percent by weight and is usually well mixed with the soil matrix. This extensive mixing makes physical separation of the moisture by filtering or centrifugation impossible. Often samples that have been cooled or frozen display increased moisture levels due to condensation of atmospheric water. In most soil samples, this water is readily adsorbed and cannot be removed. This

results in a nonsystematic source of error for moisture and wet weight explosives determination.

Plants, unlike soil samples, contain large amounts of water. The percent of total sample weight that can be attributed to water is generally between 80 and 95 percent. An accurate measurement of the plant fresh weight is required in order to provide concentration values of explosives in the plant when fresh. In order to accurately determine the fresh weight for use in the percent moisture calculation, only the water associated with the plant tissue must be present during weighing. Moisture that has adhered to the plant surface during washing or as a result of condensation could more than double the fresh plant mass. Unlike soil samples in which physical separation of this type of moisture is both difficult and unnecessary (due to the relatively low percentage of moisture observed in these samples), surface moisture on plant tissues can be dried by blotting with paper towels.

Homogenization

Method 8330 calls for homogenization of the soil samples by grinding in a mortar and pestle following air-drying. This procedure is effective for reducing particle size and increasing sample surface area. This increase in surface area serves to enhance extraction of explosives from the matrix. Unhomogenized or incompletely homogenized samples may contain particulate explosives that will affect accurate data representativeness. A dried soil sample can be ground into a fine dust that can then be subsampled without concerns about sample representativeness.

Plant tissues, due to the high organic content of the matrix, cannot be homogenized using this procedure. Effective homogenization is achieved by increasing the water content of the sample and utilizing a cooled, high-speed laboratory mill to grind the sample into a frothy paste. The resulting sample is high in water, and the particle size is greatly reduced. The various plant organs included in the sample are reduced to a homogenous slurry. This slurry can then be subsampled, while maintaining sample representativeness. Because water is added to the sample during the homogenization process, a drying step is required following homogenization of plant tissues in this manner.

Drying

Following the weighing of the wet sample, Method 8330 uses air-drying in order to allow efficient homogenization of the sample and extraction of the sample with an extraction solvent with reproducible extraction characteristics (100-percent acetonitrile, 0-percent water). Homogenization of soil samples using a mortar and pestle is facilitated by drying the sample beforehand, and the use of

identical extraction solvents allows concentrations of explosives from a number of soil samples with different moisture contents to be compared directly. The drying step is performed at room temperature due to the thermally labile nature of the explosive analytes and in the dark in order to minimize photolytic degradation. Because the majority of the water in soil samples can be easily removed by thinly spreading the soil particles in an aluminum weighing dish and air-drying in the dark for time periods from overnight to a few days, this drying technique is an efficient means of removing the majority of the water from soil samples prior to homogenization and extraction. The advantages to the analytical process proved by drying the sample in such a manner are generally thought to outweigh the negative effects of drying in such a manner. Negative effects of this process include microbial degradation of contaminants of interest during the drying step, loss in recovery due to the reduction of extraction solvent effectiveness, volatilization of analytes of interest, or sample contamination through laboratory error or vapor deposition.

The drying of plant tissues by the method utilized for soil samples is not practical for a number of reasons:

a. As mentioned above, homogenization of plant tissues is facilitated by the addition of water to the sample, and drying prior to homogenization would introduce a second drying step.

b. The large amount of water in plant tissues along with the high affinity of water for hydrophilic plant surfaces significantly increases the drying time required to remove water from the tissue matrix. This increased time has a negative effect on sample integrity due to continued plant enzymatic activity following harvesting, microbial degradation of analytes, thermal degradation of analytes, and loss of analyte due to deposition on the drying surface.

c. The physiology of many plant species has evolved the capability to retain water within the tissues through specialized organs and cell structures. As a result, water can be retained in plant tissue samples after extended periods of air-drying.

Drying of the plant tissues prior to extraction is necessary, however, in order to ensure that a water-free extraction solvent is utilized for samples containing varying amounts of water. Lyophilization or freeze-drying of the homogenized plant material serves as a time-efficient technique for water removal at this stage of the sample preparation process.

Historically, lyophilization has been used as a means of preparing samples for extraction. Recently a number of researchers have examined the impact of freeze-drying on sample integrity and analyte recovery. Dao and Friedman (1996) present a comparison of glycoalkaloid content of fresh and freeze-dried potato leaf sample preparation followed by reverse phase HPLC analysis. This analytical problem is similar to that posed by sample preparation for determination of explosives in plant tissues. They concluded that analyzing freeze-dried samples

was superior to analyzing fresh samples, with recoveries between the two methods being similar and reproducibility greater for freeze-dried samples. A comparison study employing plant tissues containing RDX has been performed (Larson, Escalon, and Parker 1997). The results of this study also indicate that freeze-drying is superior to either a fresh extraction or extraction following a nitrogen-drying procedure. Zimmerman, Kramer, and Schnable (1996) present results concerning the use of lyophilization for improved handling of *Vicia faba* leaves prior to bioassay. Their results indicated nominal loss in activity during freeze-drying. Dewanji and Matai (1996) used lyophilization as a sample preparation technique when evaluating leaf proteins in aquatic plants.

Extracting

Following removal of water from the sample, the dried material is weighed in order to provide a mass for use in calculation of the percent moisture in the original sample. A representative subsample of the homogenized material is then extracted using 100-percent acetonitrile. Both the plant matrix and the soil matrixes are extracted for 18 hr in a sonic bath equipped with water cooling. This extraction technique has proven effective at removing explosives from solid matrixes without significant losses due to thermal degradation (Jenkins 1989).

Sample Cleanup

For soil and plant matrixes, in order to remove the extraction solvent from the solid residue after extractions, the extraction vials are centrifuged, driving the solid material to the bottom. For soil samples with extensive organic matter in the acetonitrile extract, a cleanup procedure is used in which an equal volume of aqueous calcium chloride solution is added to the acetonitrile extract. This solution is allowed to sit at room temperature for 30 min during which flocculation of the organic matter occurs. The clarified solution is then filtered though a 0.45-µm syringe filter prior to analysis.

The amount of organic material that can cause analytical problems during the analysis of plant extracts is much larger than that observed in soil extracts. The majority of these compounds are not removed during flocculation with calcium chloride. In order to produce an extract that can be analyzed for low concentrations of explosives, a chromatographic cleanup step is performed using florisil and alumina as the stationary phase and 100-percent acetonitrile as the mobile phase. The resulting acetonitrile extract is then diluted with water and analyzed.

Analysis

The high performance liquid chromatographic separation and UV-visible absorbance detection of the preprocessed plant extracts by reverse phase HPLC is the same as described in Method 8330 for analyzing soil extracts. The only difference is the possible increased run time necessary to allow late-eluting plant products to exit the column before the next injection.

Organic Contaminants in Plants

Vegetation is known to act as a sink for many types of pollutants. Recently, interest has been shown in the study of the uptake of airborne contaminants by plants. Kondo et al. (1995) investigated the absorption of formaldehyde by oleander and concluded that oleander trees could act as an important sink for atmospheric formaldehyde pollution. They postulated that metabolic pathways present in the trees played an important role in absorption of airborne contaminants. Simmonich and Hites (1995) prepared a review article summarizing recent advances in methods to evaluate contminant accumulation in vegetation. Possible mechanisms for plant uptake of contaminants studied included air to plant, soil to plant, and irrigation water to plant. These scientists point out that little is known about the magnitude of vegetation's role in the fate of contaminants, and, of the studies that have been performed, a disproportionate number had to do with the uptake of pesticides by crop species. The method presented here provides a means of studying the vegetative uptake of nonvolatile contaminants associated with explosives manufacture.

Narayanan, Davis, and Erickson (1995) have studied the fate of the volatile compounds 1,1,1-trichloroethane (TCA) and trichloroethylene (TCE) in alfalfa production. They found biotransformation of the chlorinated contaminants and increasing levels of chloride in the soil where degradation occurred. This degradation was not directly attributable to plant processes, but the enhanced biological activity associated with the presence of plants appeared to facilitate degradation. Gunther, Dornberger, and Fritzche (1996) studied the effect of ryegrass on biodegradation of hydrocarbons in soil, utilizing hydrocarbons with aliphatic chains between 24 and 10 carbons, olefins, and polycyclic aromatic hydrocarbons. Accelerated disappearance of artificially applied hydrocarbons in the rhizosphere of the ryegrass was demonstrated. Schnoor et al. (1995) summarize current research into phytoremediation of organic and nutrient contaminants. A number of successful applications of phytoremediation are discussed using a number of plant species including poplars, corn, fescue, Russian olive, soybean, pine, goldenrod, and aquatic plants for contaminants including metals, hydrophobic and hydrophilic organics, and ionic contaminants. Limitations of phytoremediation are also discussed, including the possibility of bioaccumulation in plant tissues, enhanced toxicity or mobility upon transformation by plant metabolism, and the necessity of contaminant levels being low enough that plants are capable of survival before phytoremediation can be utilized.

Explosives in Plants

Studies have shown the uptake and transformation of explosives by a wide diversity of tissues and environmental systems (Pennington 1988; Harvey et al. 1990). These matrixes include mammals, plants, bacteria, soil, bioslurries, and composts. Plants grown hydroponically in TNT solutions have shown to both retain TNT and reductively transform TNT (Palazzo and Leggett 1986a,b). Considerable quantities of bound monoaminodinitrotoluenes were recovered from hydroponically grown plants using acid hydrolysis of plant material following benzene extraction (Palazzo and Leggett 1986a,b). These studies used a number of analytical procedures including air-drying, solvent extraction using methylene chloride, acetonitrile, ethyl ether and benzene with determinations using HPLC, thin-layer chromatography, and gas chromatography. The goal of this report is to provide a means of explosives analysis in plant tissues that uses the equipment normally found in laboratories performing EPA Method 8330.

There are a number of sites where concern over possible bioaccumulation of explosives is increasing. Groundwater plumes containing explosives and degradation products are increasing in size and in some cases utilized for irrigation of agricultural fields and gardens. The U.S. Army Engineer Waterways Experiment Station (WES) is currently performing a study for the U.S. Army Engineer District, Kansas City, in which the accumulation of explosives in garden vegetables (corn, corn silage, radishes, lettuce, tomatoes, and tomato plant tissues) as well as in a reference plant (yellow nutsedge (*Cyperus esculentus*)) is being studied. The preliminary results of these studies show that levels of 1,3,5-trinitrohexatriazine (RDX) in a number of plant tissues exposed to explosives are above the action level for remediative cleanup (Larson and Yost 1996; Price, Pennington, and Larson 1996). This study is ongoing and will provide information that will be useful for making risk assessment and remediation decisions.

Plants as a Remediative Tool

Innovative cost-effective remediation technologies have been proposed and are currently being utilized at the bench, pilot-plant, and full-scale level. Bioremediation technologies such as composting, land-farming, and bioslurry utilize the ability of microorganisms (algae, bacteria, and fungi) to degrade or mineralize explosives. When compared with incineration or chemical treatment, these technologies have been shown to dramatically reduce remediation costs, although they can produce large volumes of material that require finishing or dewatering to allow further handling or unregulated disposal. Bioremediation Service, Inc., is currently performing large-scale composting for explosives at Umatilla Army Depot.[1] The J. R. Simplot Company has developed a bioslurry project that

[1] Personal Communication, 1995, Patrick Faessler, Project Manager, Umatilla Explosives Composting Project, Bioremediation Services, Inc., Portland, OR.

produces large volumes of treated soil that require dewatering (Kaake 1996). To date, composting at Department of Defense installations has produced 76,455 m^3 (100,000 yd^3) of soil that retain contaminants at levels that prevent its deployment in the landscape or use as an uncontaminated horticultural matrix. Methods for determining the levels of explosives and explosive breakdown products in plants grown in this material are needed in order to ensure proper disposal of the massive volumes of finished soil material. The use of such soil material for landscaping, agricultural, or even fill material that may one day be used for agricultural purposes results in a need for a standard analytical method for determining explosives in plant tissues.

The synergism between plant and microbial processes in aerobic and anaerobic aquatic environments makes phytoremediation an attractive option for remediation. Phytoremediation can also act as an inexpensive, low-energy finishing step for other remediation processes. Nitroaromatics (TNT and transformation products) have been shown to undergo microbial degradation in aerobic conditions, while degradation of nitramines (HMX and RDX) has been observed in anaerobic systems (Comfort et al. 1995). Recent work has shown that plant enzyme-mediated explosive degradation makes phytoremediation a potential cleanup method for water and soil in wetland and upland areas (Beelen and Burris 1995; Best et al. 1996, 1997; Schnoor et al. 1995). Design of phytoremediative technologies requires a firm understanding of the fate of contaminants throughout the remediative procedure if public and regulatory acceptance is to be achieved.

There are a number of studies currently in progress at WES in which phytoremediation of explosives is being investigated. One study deals with the process of screening plants for the potential to degrade explosives; a second is designing reactor systems to facilitate this degradation; and a third is monitoring the molecular process of explosives degradation. The Tennessee Valley Authority currently has a production-level pilot study in progress investigating the degradation of explosives using a designed wetland for phytoremediation combined with anaerobic and aerobic microbial determination. All of these studies require specialized analytical techniques like the ones presented in this report.

This report addresses the important points in connection with the problem of quantifying explosives and explosive degradation products in plant matrices. The extraction of the contaminants from the matrix requires a different set of extraction techniques from those utilized for standard water and soil extractions. These matrices often contain much higher organic content than traditional soil or water matrices and, as a result, are prone to interference from biological molecules. Most matrices require some type of sample cleanup step and are discussed in the context of producing samples that can be analyzed using conventional HPLC techniques.

The ability to detect and quantify explosives and explosive degradation products in plant tissues makes a number of investigations possible. Accurate risk assessment requires knowledge of the uptake and bioaccumulation of explosives by plants. Plant tissues can be separated, and variations in contaminant levels in

separate organs can be determined. The differences in the concentrations and contaminants found in the different parts of the plant are useful in identifying where the explosive degradation is occurring and which compounds are the most mobile inside the plant itself. This method provides those interested in utilizing plants to clean up explosive contamination with a tool for determining the degradative pathway followed by the contaminants and provides insight on the ultimate fate of the chemicals in the environment.

2 Experimental Techniques

The EPA Standard Method 8330 (EPA 1992; Jenkins 1989) for the analysis of explosives (analytes listed in Table 1) in water and soil samples is used as a base for the detection of nitroaromatics, nitramines, and their degradation products in plant samples. Quantitation of these analytes requires matrix-specific sample preparation, separation by reversed phase HPLC, and ultraviolet detection. The specifics of the method are outlined below.

Analytical System

The equipment used in sample preparation is as follows: centrifuge and centrifuge tubes (3,000 rpm), syringes and filters, volumetric flasks, automatic pipettes, and autosampler vials.

The HPLC system consists of a Waters 610 Fluid Unit Pump capable of achieving 6,000 psi, a Waters 717 plus Autosampler including a 200-µL loop injector, a Waters 486 Tunable UV Absorbance Detector monitored at 245 nm, and Millenium 2.1 Chromatography Software (Waters Chromatography Division, Milford, MA). A Supelco LC-18 reverse phase HPLC column 25 cm by 4.6 mm (5 µm), Catalog No. 5-8298, is used as the primary column; a Supelco LC-CN reverse phase HPLC column 25 cm by 4.6 mm (5 µm), Catalog No. 5-8231, is used as a confirmation column. The appropriate precolumn, Novapak C-18, Catalog No. WAT015220, or Novapak CN, Catalog No. WAT020800 (Waters Chromatography Division, Milford, MA), is utilized. A Cera Column Heater 250 set at 30 °C, Catalog No. 282-0252 (Cera, Inc., Baldwin Park, CA), is used to ameliorate retention time shifts due to changes in room temperature.

Sonication extractions are performed with a temperature-controlled ultrasonic bath where the temperature does not exceed 30 °C. The filtration system used for sample preparation consists of a disposable LurLoc syringe and disposable 0.50-µm Teflon filter cartridges.

All standards and test solutions were prepared from Standard Analytical Reference Materials, which were obtained from the U.S. Army Environmental

Table 1
Target Compounds

Compound	Abbreviation	CAS[1] No.
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	TNB	99-35-4
1,3-Dinitrobenzene	DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-A-DNT	1946-51-0
2-Amino-4,6-dinitrotoluene	2-A-DNT	355-72-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

[1] Chemical Abstract Service.

Center, Aberdeen Proving Ground, Maryland. HPLC-grade acetonitrile from Baker was used in the preparation of all stock standards. HPLC-grade methanol from Baker was used to prepare the HPLC eluent. The HPLC mobile phase (1:1 (v/v) methanol/reagent water) is prepared by combining 500 mL of each, using graduated cylinders. Vacuum Filtration System from Millipore with 0.22-μm filters is used to degas and to remove particulate matter. Unless otherwise indicated, all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.

Sample Handling

The determination of explosives and explosive degradation products in complex matrices such as plant tissues requires precise and reproducible sample handling. Sample processing is taken through a series of actions: sample collection, storage, transport, preparation, homogenization, drying, extraction, cleanup, and analysis. The determination of the concentrations of explosives and explosive degradation products is not made until the last step in the process.

The first step in the analysis process is sample collection. A number of biases can be introduced during sample collection.

a. During harvesting, contaminated soil is included with the plant tissue sample. This is especially problematic when analyzing subsurface plant tissues such as root tissues.

b. Aquatic plants harvested from water contaminated with explosives must be thoroughly rinsed with uncontaminated water in order to remove explosives associated with the water on the plant surface.

c. Plant tissues must be rinsed, blotted dry, sealed, and frozen immediately after harvesting. Leaving plant tissues at room temperature allows microbial growth, plant necropsy, and drying to occur. All of these can change the concentrations of explosives and explosives degradation products in the tissue.

Plant storage during transport and in the laboratory prior to analysis is another important cause of loss of sample integrity. Generally, freezing is the preferred method for plant storage. Conventional freezers (-15 to -20 °C) are utilized for this purpose. Maintenance of plant material below the freezing point of water is expected to slow microbial action sufficiently to retain sample integrity over short periods of time.

Preparation of Plant Samples

In order to achieve optimal extraction of explosives contained in plant tissues, the matrix is homogenized using a high-speed laboratory mill that produces a high surface area sample for extraction. The rupture of the cellular matrix of plant material by high-speed milling produces extensive contact between the plant material and the acetonitrile extraction solvent. Any dissection of plant material must occur prior to homogenization. Addition of deionized water to the cut or chopped plant material facilitates the formation of homogenized material. The temperature of the grinding mill must be monitored and if necessary cooled with an ice/water slurry during homogenization.

Frozen plant samples are allowed to come to room temperature, and a representative subsample of the plant tissue is taken and placed onto a clean paper towel to remove any excess moisture. An appropriate amount of the plant species (Table 2) is weighed (±0.5 g) and recorded. Using scissors and/or a knife, the samples are cut into small (less than 1-cm) pieces. The cut up sample is placed into a homogenizing chamber (ES-115A). Milli-Q water is added to just cover the top of the sample, and the mixture is homogenized using a sawtooth generator probe, beginning at 500 rpm. Once 500 rpm has been reached, the speed is increased in intervals of 2,500 rpm, 5,000 rpm, and 7,500 rpm. If the sample is not a frothy paste, homogenization is continued at 10,000 rpm. After homogenizing the sample to a frothy paste, the sample is poured into the 120-mL Labconco freeze-drier flask. The flask is covered with parafilm and placed in the freezer until frozen (approximately 3 to 4 hr). The generator probe is cleaned between samples by multiple rinses of the generator probe, homogenizing Milli-Q water in a flask.

The freeze-dryer cooling unit is activated; when the temperature reads less than -40 °C, the vacuum pump is activated. A freeze-drier filter is inserted between the

Table 2
Plant Species and Masses Extracted

Plant Species	Fresh Weight, g
Cyperus	5.00
Aquatics	5.00
Corn kernels	10.0
Corn silage	10.0
Tomato fruit	20.0

freeze-drier adapter and rubber top. The rubber top is placed onto the freeze-drier flask containing the frozen sample. The exposed adapter end is inserted into the valve on the freeze-drier condenser. The plastic valve stem is turned to the "vacuum" position. Once the vacuum reading has dropped to below 200, the next sample is added. The sample is removed from the freeze-drier when the flask is no longer cool to touch and no ice chunks or crystals are left in the sample (approximately 2 days to dry most samples). The flask is removed from the freeze-drier by turning the plastic valve stem to the "vent" position. The rubber top is removed from the sample flask.

An aluminum disposable weighing dish is placed onto the balance and tared. The freeze-dried sample is placed into the disposable weigh dish. The dry weight of the freeze-dried sample is recorded. The freeze-dried sample (0.25 g) is weighed (record weight) into a 20-mL amber glass vial with a Teflon-lined cap. Samples and associated quality control samples are spiked with spiking solutions. A matrix spike is prepared by adding 0.100 mL of an acetonitrile solution containing 100 mg/L HMX, RDX, TNB, TNT, 4-A-DNT, and 2,4-DNT to 0.25 g of freeze-dried sample. Acetonitrile (10.0 mL) is added volumetrically. Using a vortex mixer, the sample is swirled for 1 min and placed in a cooled ultrasonic bath for 18 hr.

After sonication, the sample is placed into the centrifuge at 2,500 rpm for 5 min and allowed to sit for approximately 1 hr. Supernatant (5 mL) is removed using a 5-mL pipet with disposable tips and placed in a 20-mL vial. Filter columns to clean up all of the samples are prepared by the following procedure:

a. Placing a small piece of glass wool into a 146-mm (5-3/4-in.) glass disposable pipette.

b. Placing 0.5 g of florisil into the pipette.

c. Place 0.5 g of alumina into pipette (on top of florisil).

d. Rinsing the filter column with 5 mL of acetonitrile. Discarding the filtrate.

The supernatant is added to the cleanup column using a glass disposable pipette, and the filtrate is collected in a 20-mL glass vial. After the supernatant has completely passed through the cleanup column, an additional 5 mL of acetonitrile is passed through the column and collected in the 20-mL glass vial containing the cleaned up supernatant. The cleaned up supernatant is vortexed for 1 min. The cleaned up supernatant (2 mL) is transferred, using a 5-mL pipet with disposable tips, into a 20-mL vial. Milli-Q water (2 mL) is added volumetrically to the same 20-mL vial. Using a disposable glass Pasteur pipet, the supernatant is placed in a disposable syringe and filtered through a 0.50-μm Teflon filter attached to the syringe. The first 1 mL is discarded and the remainder retained in a 10-mL glass Teflon-capped vial for HPLC analysis.

Preparation of Plant Species for MDL Study

In order to perform the study of method detection limits (MDL) for the plant species, corn, corn silage, tomato, cyperus, and radish reference samples of unexposed plant tissues were prepared for spiking. Frozen plant tissue (100 g) was allowed to warm to room temperature. The tissue was cut into small pieces using a knife or scissors and homogenized as described above. The homogenized tissue was then placed in a freeze-drying flask and stored in a freezer for 5 hr prior to lyophilization. The samples were freeze-dried for 48 hr. The resulting freeze-dried plant material was weighed to determine dry weight and 0.25 g of dried material placed in 20-mL amber vials. Acetonitrile (10 mL) spiked with explosive analytes at 0.125 ppm was added to the samples. The samples were extracted, cleaned up by liquid chromatography, and analyzed as described above.

Interferences

To this point, no single significant interference is identified for the determination of explosives in plant samples. As mentioned above, analysis of explosives from plant sample matrices that contain large amounts of extractable compounds that absorb at 245 nm may result in high background absorbances, unidentified interfering peaks, or analyte decomposition prior to detection. Accurate explosives measurement by this method may prove to be impossible in these cases. Many of these interferences can be kept to a minimum through the use of rigorously clean reagents and sample processing equipment.

Safety

The nitramine and nitroaromatic analytes are suspected carcinogens. A number of the degradation products of nitroaromatics exhibit enhanced toxicity compared with the parent compounds. The nitrosoamines are a class of organic contaminants that are known carcinogens (Baumgarten 1982). Good laboratory

technique and protective equipment are required during the entire analysis as a result of both the safety risks associated with the analyte and the need to minimize background current arising from contamination. Protective equipment includes impermeable gloves, safety glasses, and fume hoods. Standards and eluents should be disposed of in accordance with approved regulatory practices.

3 Discussion

The performance of the method developed for the determination of explosives in plant tissues was investigated on a number of levels. Evaluations were made to determine the efficacy of each step in the analytical process prior to adoption, the extent of the information obtainable by the method with regards to the fate of explosive contamination in plants, the extent of the usefulness of the method by a range of plant tissues, the detection limits associated with the use of the method, and the range of explosive concentration the method is capable of determining. These method performance factors are discussed in detail below.

Homogenization

The homogenization of plant tissues with water in a cooled high-speed laboratory mill provides a means of reducing particle size, increasing extractable surface area as well as producing a liquefied sample that may be subsampled without corrupting sample representativeness. Figure 1 displays a scanning electron microscopy image of homogenized *Cyperus esculentus* tissue. The plant material in this micrograph is generally fibrous in nature, maintaining a large degree of its cellular structure. The amorphous material in this micrograph appears to be the walls of cells that were lysed during the homogenization process. The reduction in particle size during homogenization is achieved through the mechanical shearing of the tissue by the high-speed rotor blade, as can be seen at the end of the fiber shown in Figure 2. The shearing of this fiber at an angle across the length of the fiber is a result of the mechanical action of the rotor. The cells at the edges of the fiber are no longer intact, and extracellular material can be observed at the edges of the particle. The cells that make up the bulk of the fiber are still intact. Homogenization of the plant material by this method results in a liquefied sample made up of small particles of plant tissues providing a high surface area for solvent extraction.

Drying

Whereas in Method 8330 for soil, homogenization of soil samples using a mortar and pestle is facilitated by drying the sample beforehand, and this drying

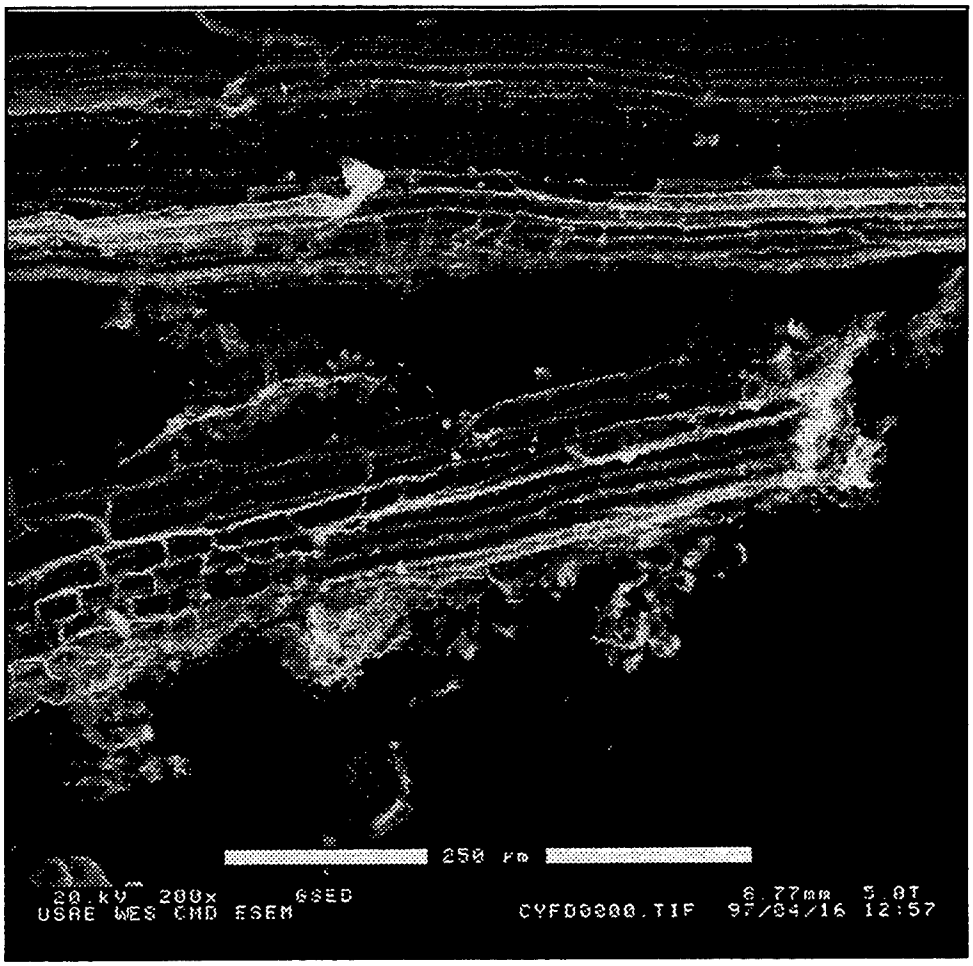

Figure 1. Homogenized and lyophilized tissues prior to extraction

allows the use of identical extraction solvents for samples with varied concentrations of explosives and geographical origins, the drying of plant tissues by the method used for soil samples is not practical for reasons addressed above. Drying of the plant tissue prior to extraction is necessary, however, in order to ensure that a water-free extraction solvent is used. Lyophilization or freeze-drying of the homogenized plant material serves as a time-efficient technique for water removal at this stage of the sample preparation process.

There are a number of advantages gained by freeze-drying plant samples prior to analysis:

a. Removal of water further lyses cells, decreases particle size, and increases surface area for extraction of explosives.

b. The rate of drying is increased greatly over the common EPA Method 8330 practice of air-drying, decreasing the time during which microbial alteration can occur.

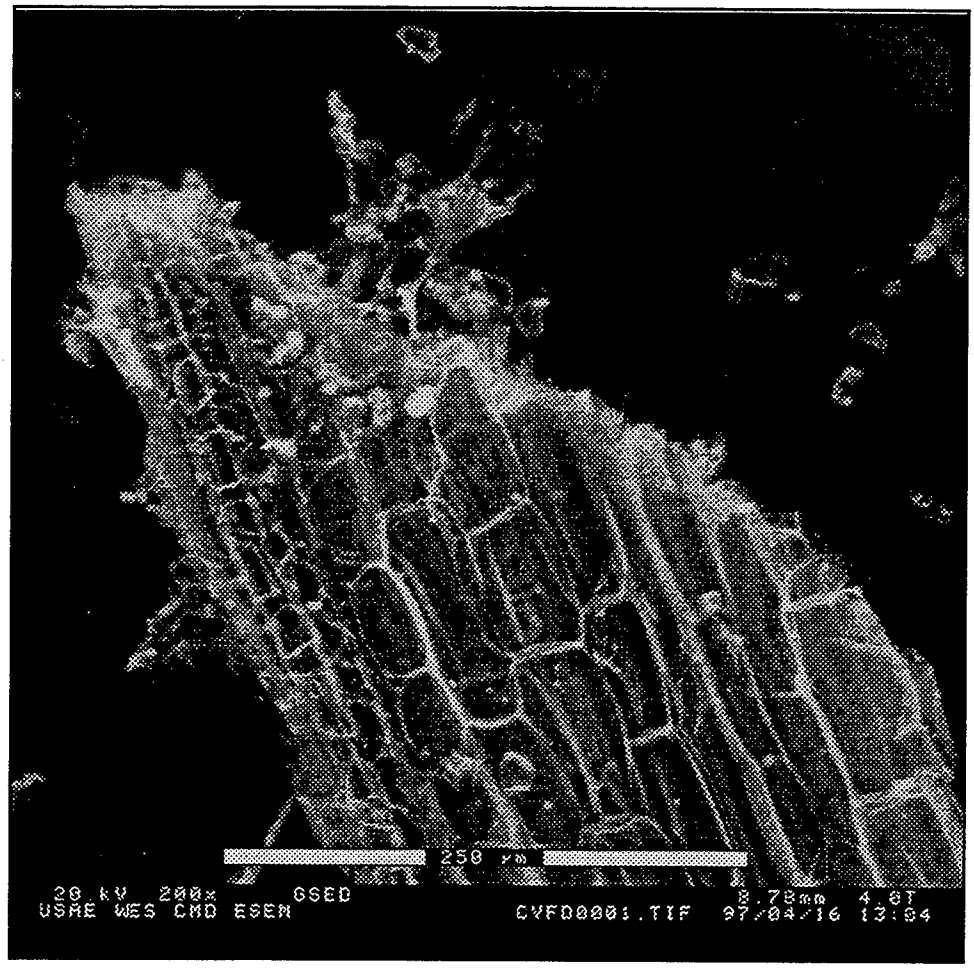

Figure 2. Homogenized and lyophilized tissues following extraction

c. The sample stays cold (0 °C) until it is completely dry, reducing thermal degradation and slowing or stopping microbial and enzymatic activity.

d. Freeze-drying stops enzyme-catalyzed, wound-induced, and moisture-dependent sample changes.

e. Freeze-drying allows storage and transportation of samples for analysis.

f. Freeze-drying produces a homogeneous sample for subsampling prior to extraction.

g. Elimination of water allows a uniform extraction solvent to be used from sample to sample.

These advantages of maintaining sample integrity, sample handling, and extract ability are generally thought to outweigh losses associated with possible analyte volatilization or transformation during the drying process.

Extracting

The sonication of the lyophilized tissues in 100-percent acetonitrile extraction solvent for 18 hr allows extensive contact between the tissue surface and extraction solvent. The penetration of the extraction solvent into the solid matrix allows the high solubility of the explosives analyte in acetonitrile to form an osmotic pressure gradient that draws the analyte from the tissue to the extraction solvent. Visual examination of the plant tissue before and after extraction shows a difference in color of the solid matrix. Prior to extraction, the dried tissue is green in color; after sonication, the dried sample appears as a lighter green. This is due to the extraction of the chlorophyll-based plant pigments during the sonication.

In order to understand the physical transformations of the tissue matrix during sonication, dried samples were examined using environmental scanning electron microscopy after extraction. Figure 1 displays representative dried plant tissue particles following sonic extraction at 200x magnification. This image displays a number of features characteristic of tissue particles before and after extraction. The end of one of the long fibers of plant tissue observed after homogenization and lyophilization is presented in Figure 1. The sample is made up of long, roughly circular fibers that maintain the cellular arrangement demonstrating tissue-level characteristics. Generally, the areas of the fibers where disruption has occurred are the ends of the long fibers. The roughness at the ends of these fibers is most likely due to the mechanical shearing that occurs during the homogenization process. It is at the ends of the fibers that individual cells are observed to be cleaved.

Figure 2 contains short fragments of fibers. The length of the fibers in the postsonication tissues is substantially less than before sonication. These fibers do not maintain the cellular arrangement at the tissue level. While Figure 1 showed disruption at the end of the fiber, Figure 2 shows that, after sonication, the disruption in the tissue is extensive at the ends and the sides of the fibers. Subcellular material is present in Figure 2. This material appears to be the cell wall membranes of disrupted cells. The reduction in fiber length, disruption of tissue-level cellular organization, and the subcellular membrane material all point to lysing of cells, increase in matrix surface area, and extensive solvent penetration into the solid matrix. This can be attributed to attack of the matrix by the extraction solvent that is aided by the vibrational energy provided by the sonic bath.

Sample Cleanup

Because of the lack of specificity of performing quantitation with absorbance at 245 nm, a chromatographic cleanup of the extract is required in order to remove interfering compounds that absorb at this wavelength. The chromatographic cleanup techniques use a 100-percent acetonitrile eluent and a florisil/alumina

stationary phase. The recovery of spikes following cleanup is greater than 90 percent with the exception of HMX for which losses up to 70 percent are observed. The stationary phase does remove large amounts of the interfering plant compounds, providing an extract for HPLC analysis that is relatively interference free.

Analysis

Figure 3 shows chromatograms acquired by spiking homogenized corn fruit with purified explosives standards at 26.3 mg/kg fresh weight, 80 mg/kg dry weight. These solutions were prepared by homogenizing corn by the procedure listed above, freezing, freeze-drying, extracting, cleaning up, and analyzing the sample by reverse phase HPLC. The elution order on the polar confirmation column, CN derivitized silica, is different from that observed on the C18 column for the explosives in the standard mixture. Generally the nonpolar C18 analytical column is considered to allow the most polar compounds to elute first and the nonpolar compounds to elute later. The CN column contains silica, coated by a more polar (relative to the C18) cyanide derivative. As a result, the polar compounds are retained on this column, while the nonpolar compounds elute more quickly. The use of two columns on which the same compounds exhibit large differences in retention factors serves to confirm the peak identified on the primary column by ensuring the same compound is identified at the same concentration and distinctive retention time on the second column. The likelihood of an unknown interfering peak matching retention times on a single column is quite high, but a significantly different molecular compound is not likely to have identical retention characteristics on two columns with dissimilar solid phases. As stated above, interferences are common when matrices containing high levels of biological material are analyzed, and utilization of a confirmation column is necessary in order to produce significant results.

Figure 4 provides an example of a chromatogram showing the separation and the determination of RDX on a C18 analytical column in a number of plant tissues that were exposed to contaminated water (0.100 ppm RDX) and soil (5.8 ppm RDX). It should be noted in Figure 4 and the following chromatograms that a number of peaks are present that are not attributed to explosives contamination. Each of these compounds is a potential interference on one or both of the columns used to separate the explosives from the matrix as well as from other environmental contaminants. Solid and liquid samples with high organic carbon are more prone to these interferences than traditional soil and water samples. The necessity of the use of a confirmation column for each positive analyte identification on the primary column is even greater than in EPA Method 8330 (EPA 1992). Another necessity of performing HPLC analysis on plant and compost samples for explosives is the use of representative blank samples. Samples that are known not to have been exposed to explosives can be used to help eliminate incorrect peak identifications. No representative reference material or set of reference materials is currently available for this purpose. As a result, if a representative blank matrix is to be available, it is imperative that those who design studies consult

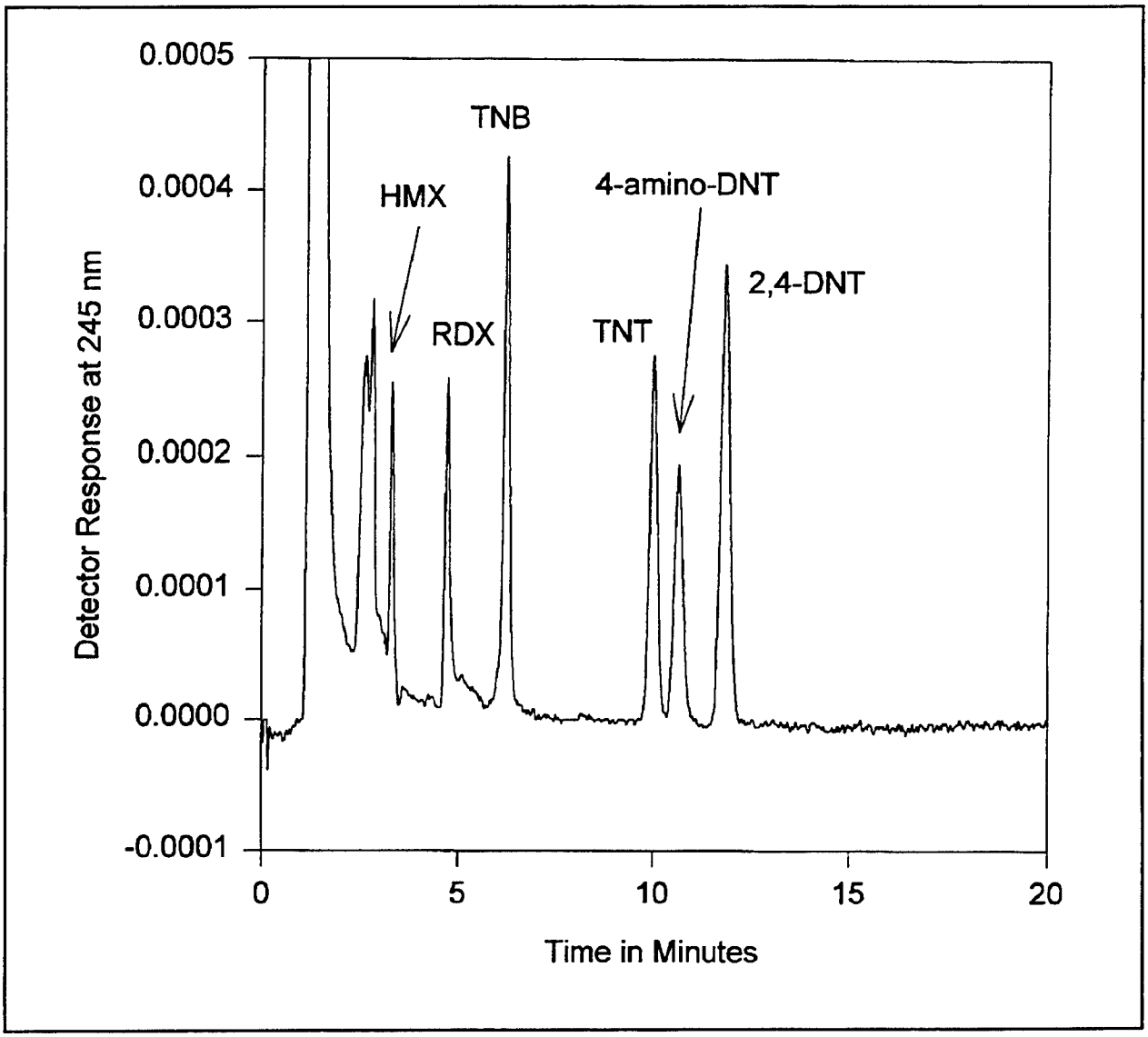

Figure 3. Corn extract spiked with explosives standards (at 1 ppm)

with the analytical team making explosives determinations. As shown in the radish chromatogram in Figure 4, the peaks at 4.5, 5.8, and 7.7 min are observed in the exposed radish, and they are also observed in an unexposed radish sample that has undergone an identical sample preparation and cleanup. With this information, the analyst can easily discount these peaks as noninterfering, naturally occurring compounds inherent to the radish matrix. It should be noted that the examples supplied in Figure 4 contain high levels of explosives contamination. When the RDX concentration is considerably smaller, more interferences become important.

Interferences from naturally occurring plant compounds that have significant absorbance at the detection wavelength for explosives can result in false positives. When performing HPLC analysis on plants directly after extraction, these

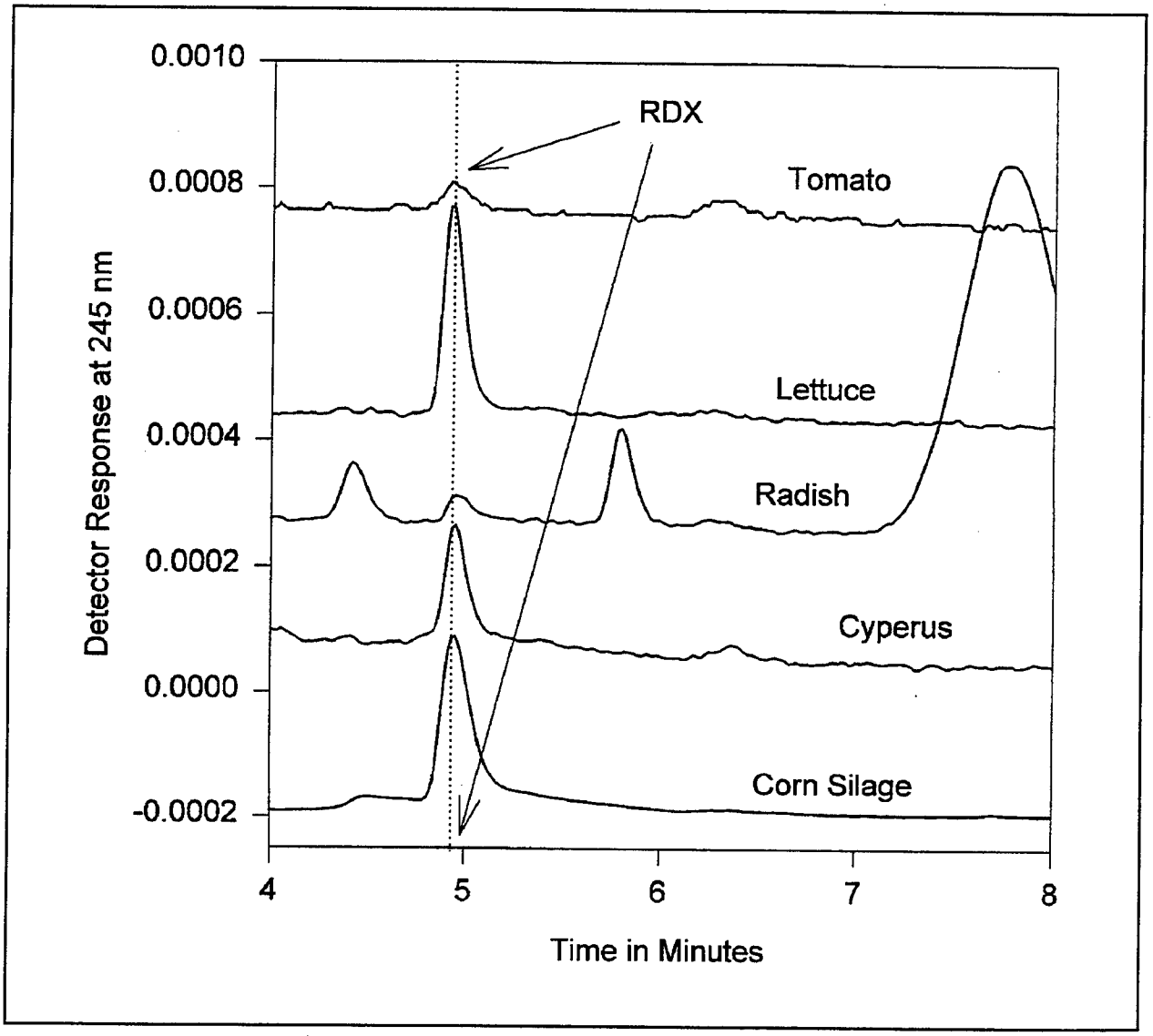

Figure 4. RDX detected in garden crops

interferences make reliable detection and quantitation of explosives difficult or impossible in most plants. As a result, a liquid chromatographic cleanup procedure utilizing florisil and alumina stationary phases and a highly polar organic solvent mobile phase is used to remove a large portion of these interferences prior to analysis. Plant cleanup by this process also removes the majority of the large biologically based compounds that are immobile on the analytical columns and cause column fouling, inconsistent retention times, and poor peak shapes. Only HMX, the most polar of the analytes listed in Table 1, is retained on the cleanup column under the conditions utilized.

Figure 5 shows a chromatogram obtained from a pilot-scale flow-through aquatic plant phytoremediation process (Medina and Larson 1996). The plant used in this study was parrot feather (*Myriophyllum aquaticum*). Concentrations

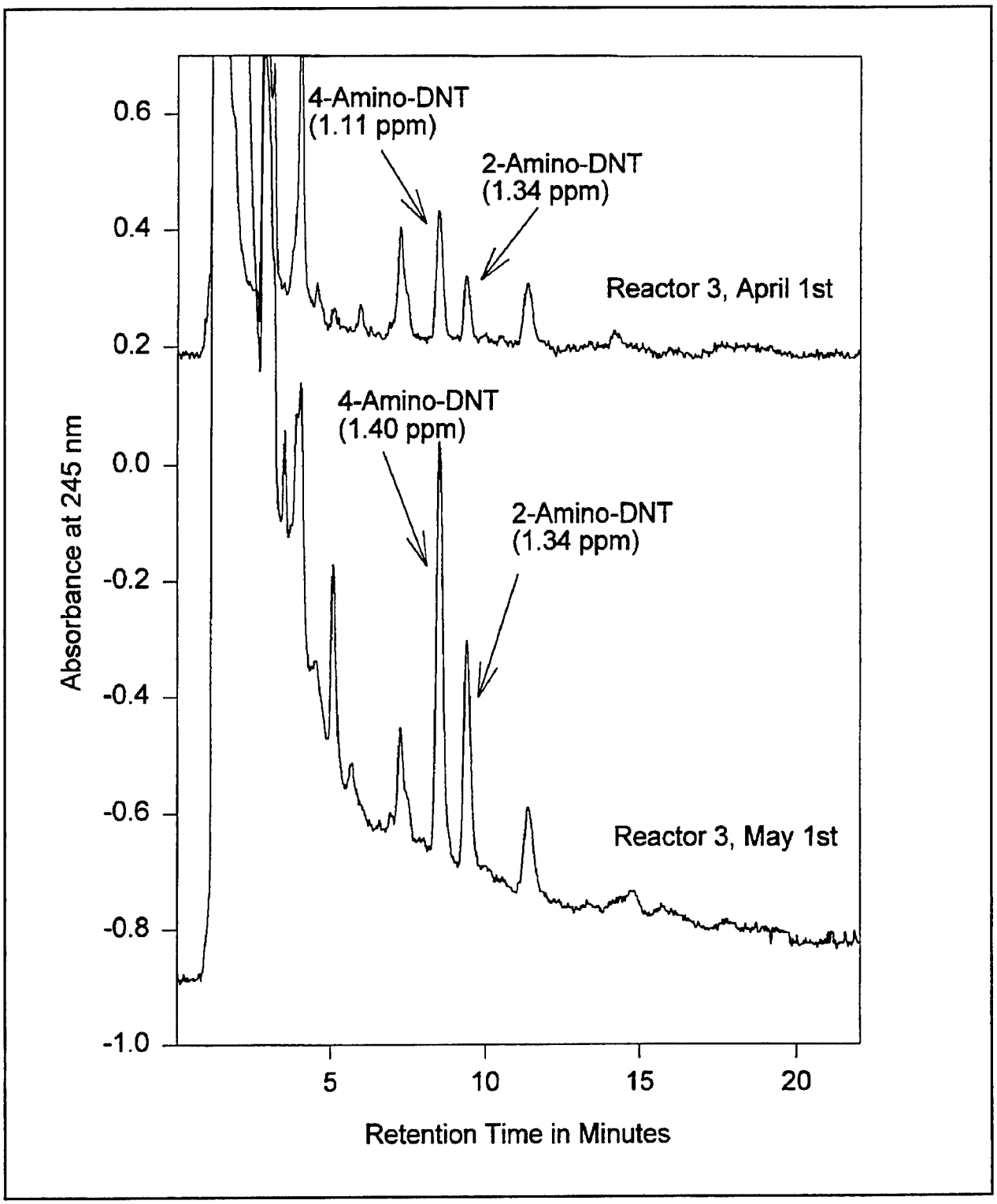

Figure 5. Parrot feather tissue on CN column

of TNT in the flow-through reactor were maintained at 2 ppm for a period of 2 months. The plants were then washed, frozen, shipped to WES, and analyzed using the process described above. As can be seen, two explosive-based contaminants are present in the tissue, 4-aminodinitrotoluene and 2-aminodinitrotoluene. The concentrations of these compounds in the tissue on a dry weight basis was 1.11 and 1.34 ppm after 30 days of exposure and 1.40 and 1.34 ppm after 60 days of exposure.

Figures 6 and 7 display chromatograms of parrot feather tissue obtained using C18 and CN analytical columns. These chromatograms from the tissue organs were exposed to TNT in the same style flow-through reactor described above with aqueous TNT concentrations of approximately 5 ppm. The plant material was separated into three fractions prior to homogenization. In the leaf tissue, the concentrations of TNT, 4-A-DNT, and 2-A-DNT, respectively, are 3.68, 10.24, and 15.6 ppm on a dry weight basis. In the stem tissue, the concentrations of TNT, 4-A-DNT, and 2-A-DNT, respectively, were 0.16, 0.448, and 20.64 ppm. In the root tissue, the concentrations of TNT, 4-A-DNT, and 2-A-DNT, respectively, were 0.499, 21.8, and 58.9 ppm.

Figures 8-14 display chromatograms of extracts from a number of plant species. As can be seen in these figures, there is a large amount of variability in the ability of this method to determine RDX in various plant tissues. Some tissues like corn silage (Figure 8), *Cyperus esculentus* (Figure 9), and lettuce (Figure 14) have high uptakes of RDX, producing large peaks that are relatively interference free. This allows quantitative and qualitative determination of RDX on both columns, providing excellent analytical accuracy and reliability. Other tissue types do not contain large amounts of RDX even when exposed to high concentrations in soil and irrigation water. Examples of these tissue types are radish (Figure 13) and red and green tomato fruit (Figures 10 and 11). Following sample preparation, these tissues provide an extract that is relatively free of interferences. The low levels of RDX found in these tissues make quantitation and confirmation difficult. Some tissue types do not have sufficient removal of interferences for quantitative determination of RDX to be performed. An example of such a matrix is the tomato plant (Figure 12). Interferences from naturally occurring plant compounds make it difficult to determine RDX in these types of matrixes. Generally, it is possible to see explosives in most garden vegetable species at detection limits that are similar to those for determining explosives in soils.

Table 3 below summarizes the analytical capabilities for a number of tissue types. The column labeled as (C18[RDX[-CN[RDX])/CN[RDX] is presented in order to illustrate the reproducibility of analyte quantitation on the two columns. The number is close to zero when the values returned from peaks at the appropriate retention times on the two columns are nearly identical (e.g., corn silage, cyperus, red tomato fruit, and lettuce). Large positive numbers indicate that the number returned from analysis on the C18 column is significantly greater than the one returned from CN column (e.g., radish and green tomato fruit). This points toward a natural interference at the RDX retention time on the C18 column, which is artificially increasing the peak height attributed to that analyte. A large negative

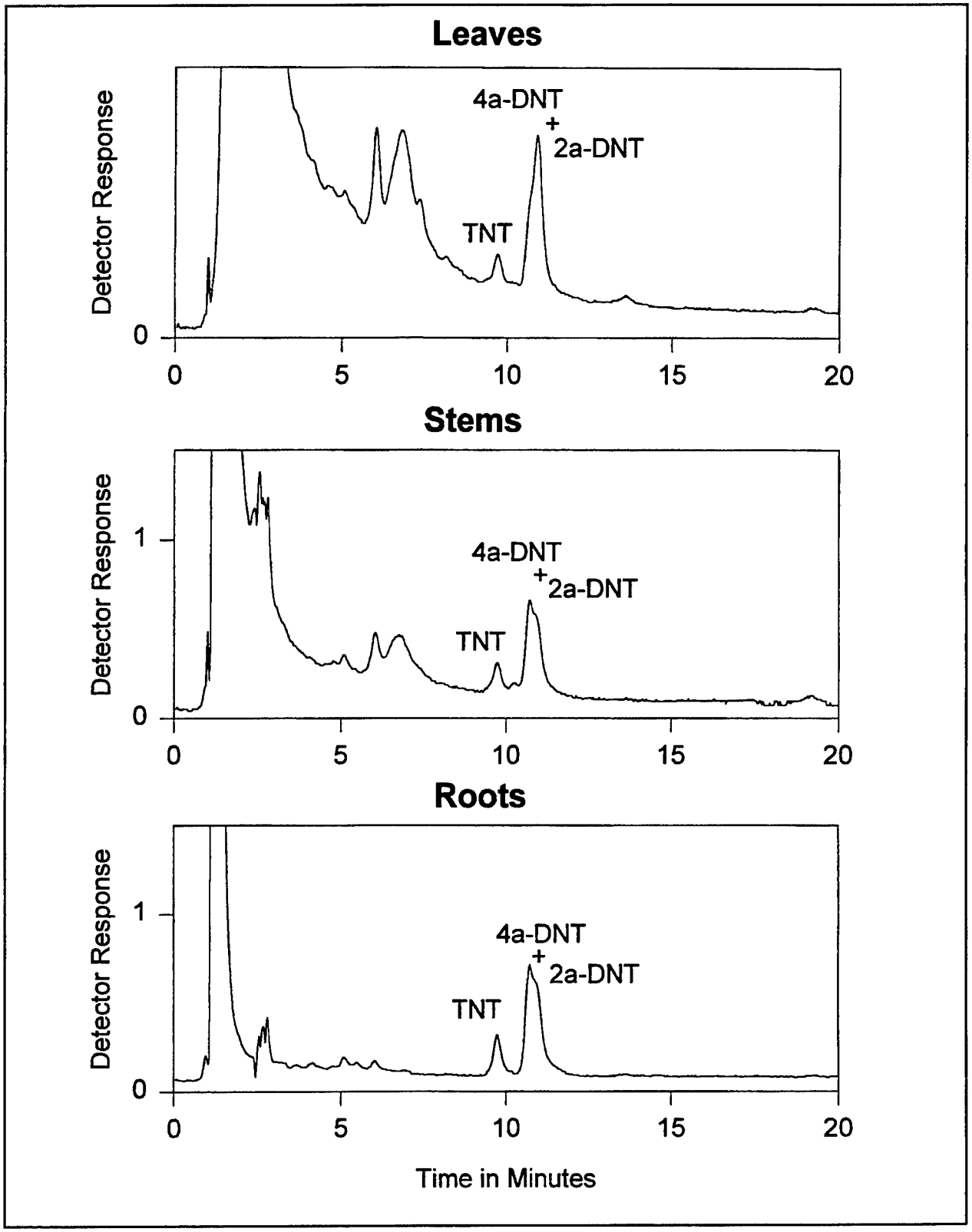

Figure 6. Parrot feather, by tissue type, on C18 column

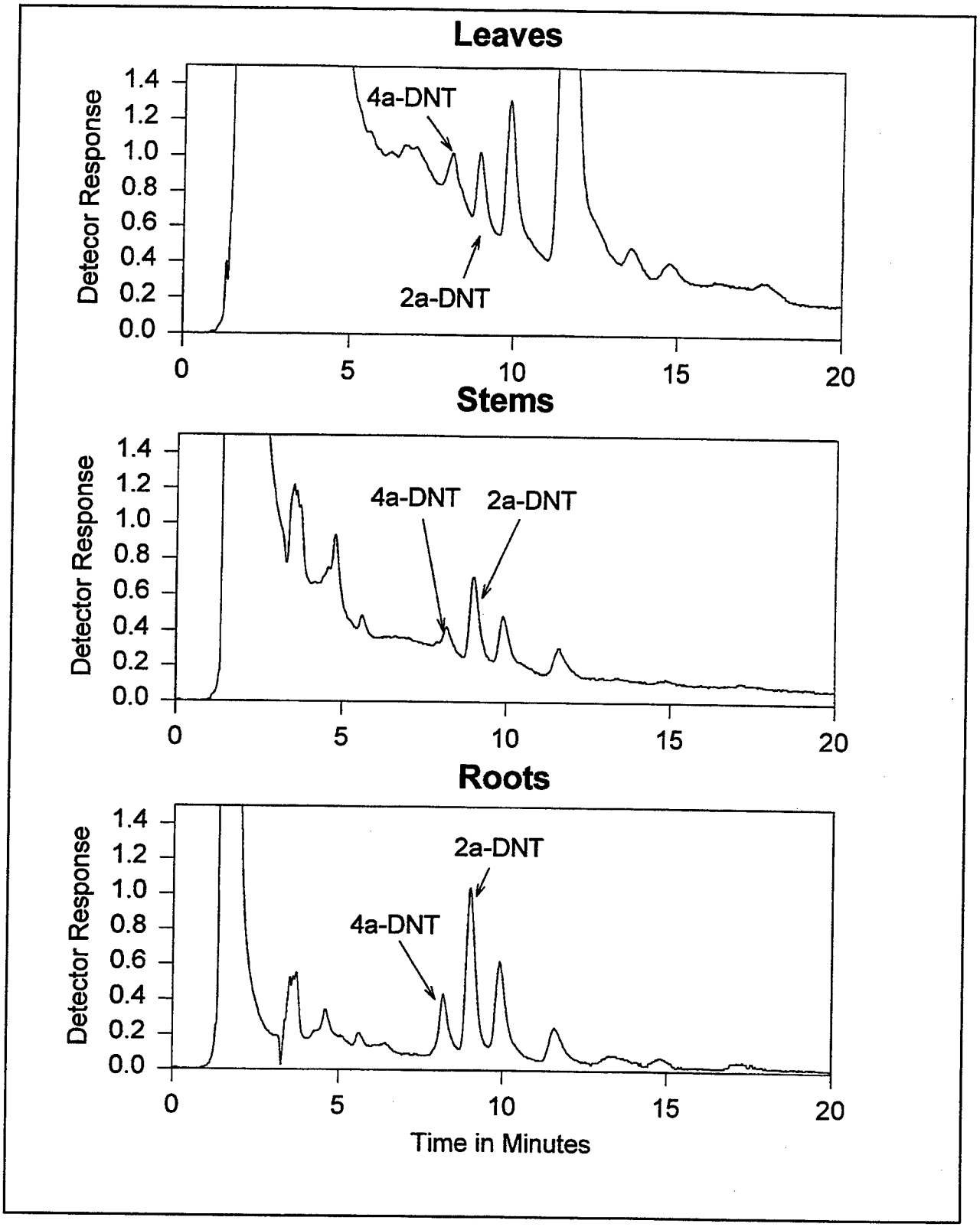

Figure 7. Parrot feather, by tissue type, on CN column

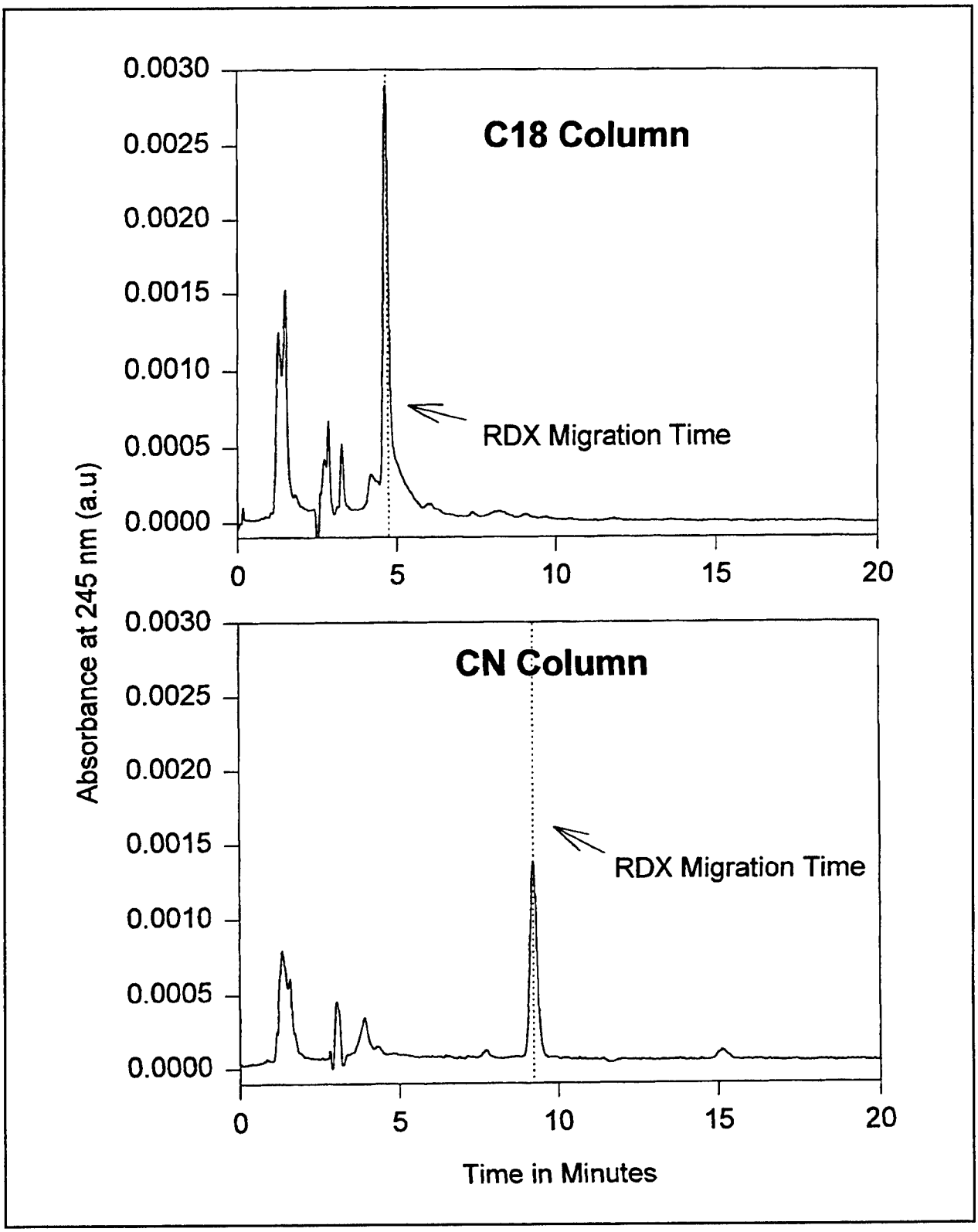

Figure 8. Chromatograms of RDX containing corn silage

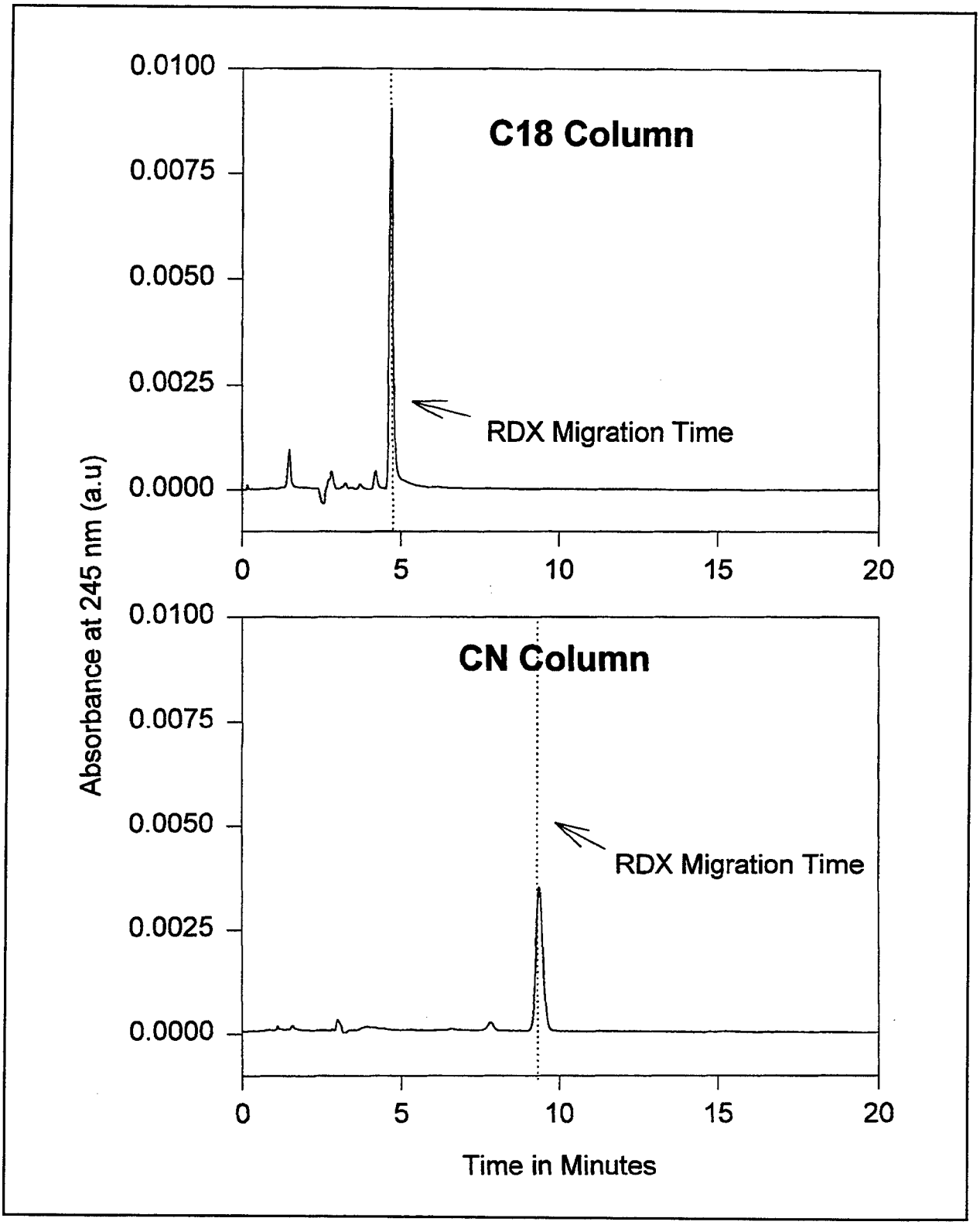

Figure 9. Chromatograms of RDX containing *Cyperus esculentus*

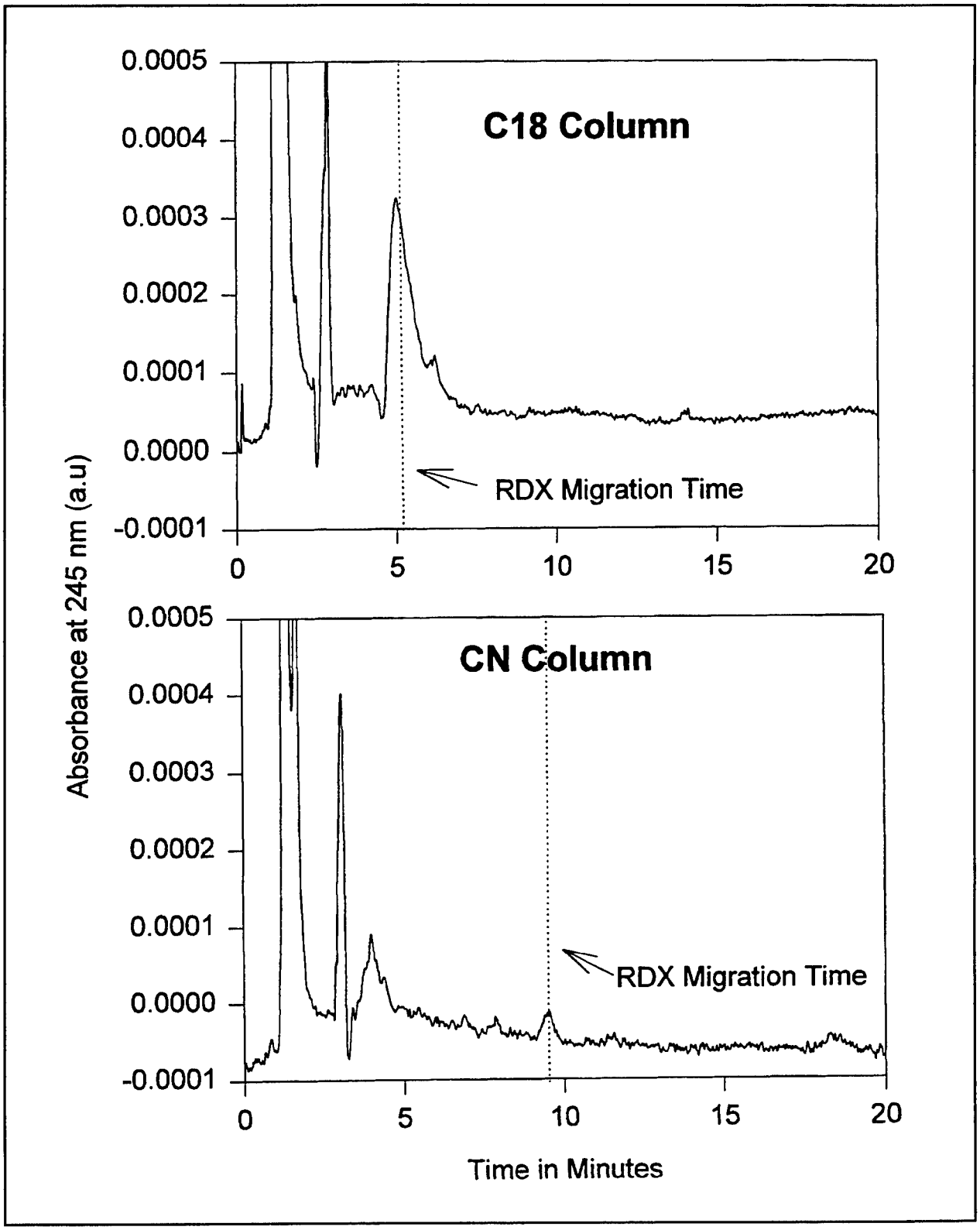

Figure 10. Chromatograms of RDX containing green tomato fruit

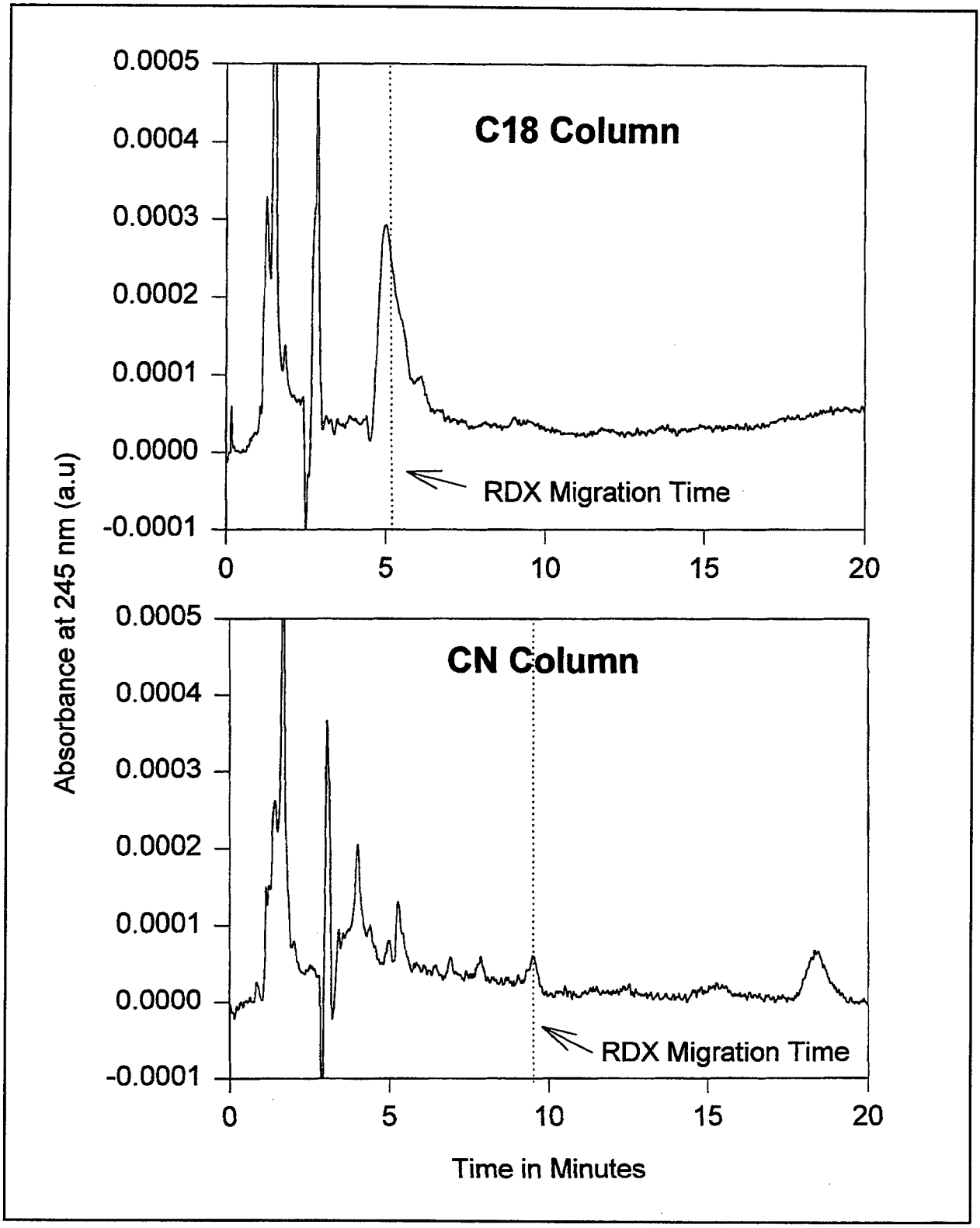

Figure 11. Chromatograms of RDX containing red tomato fruit

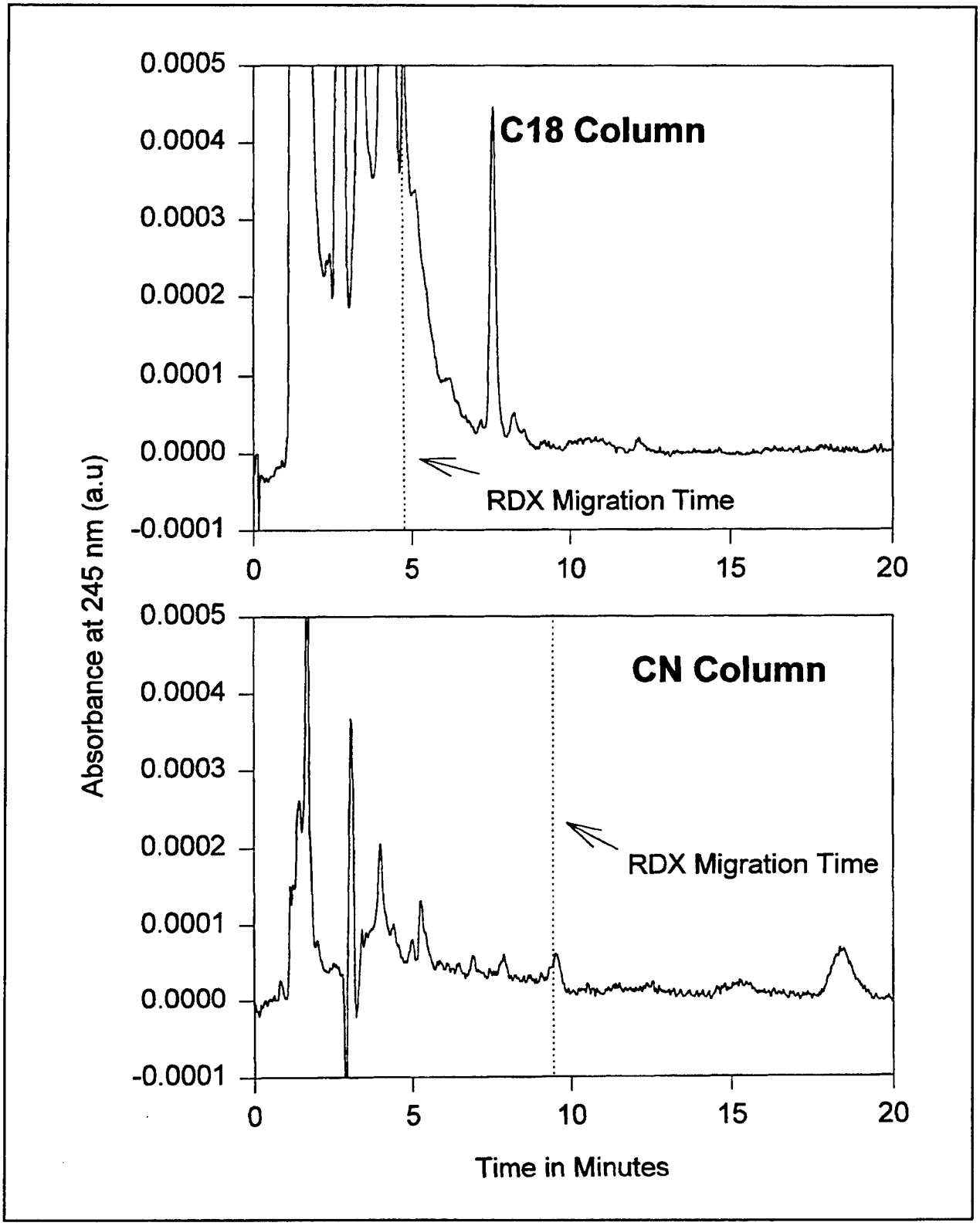

Figure 12. Chromatograms of RDX containing tomato plant

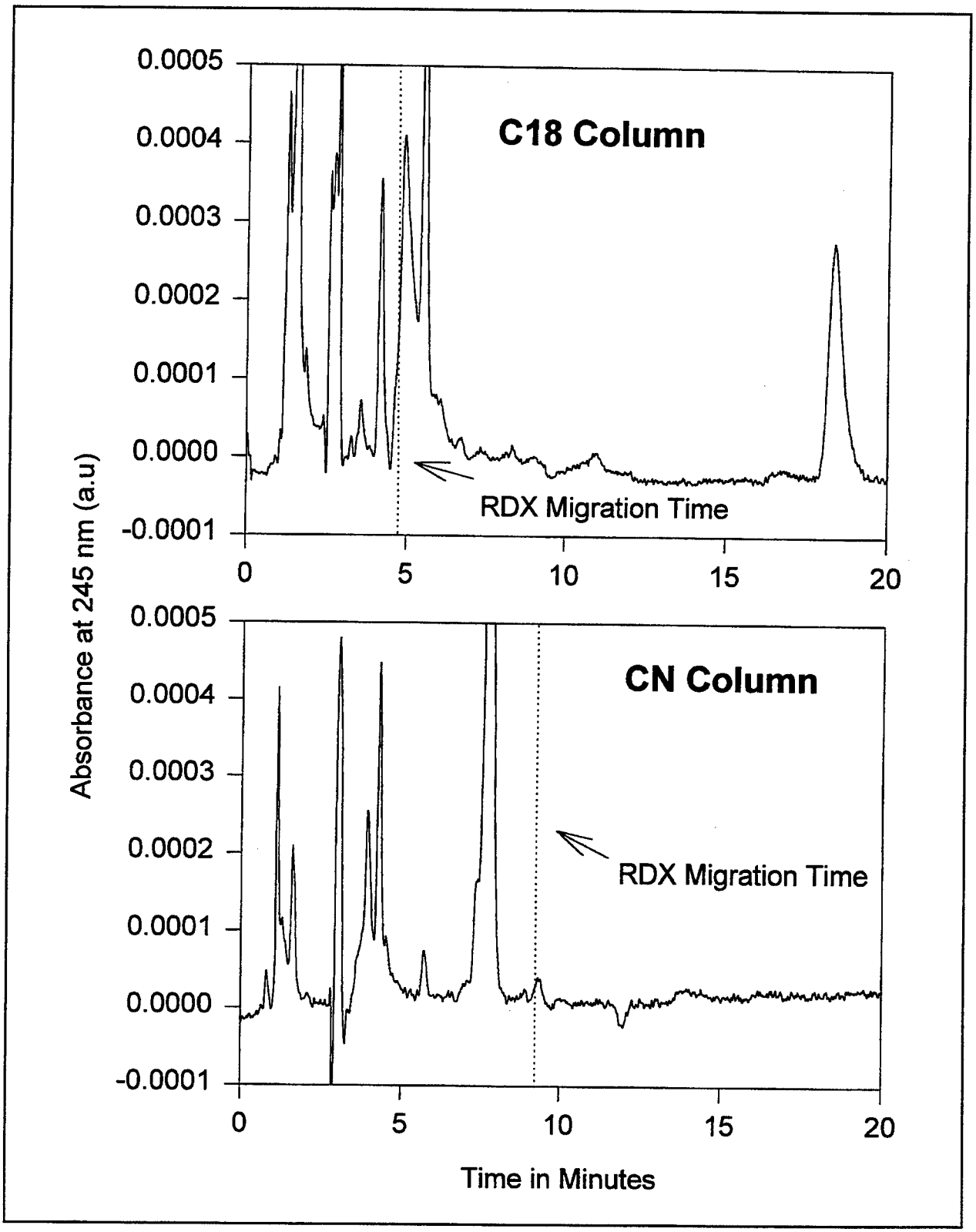

Figure 13. Chromatograms of RDX containing radish root

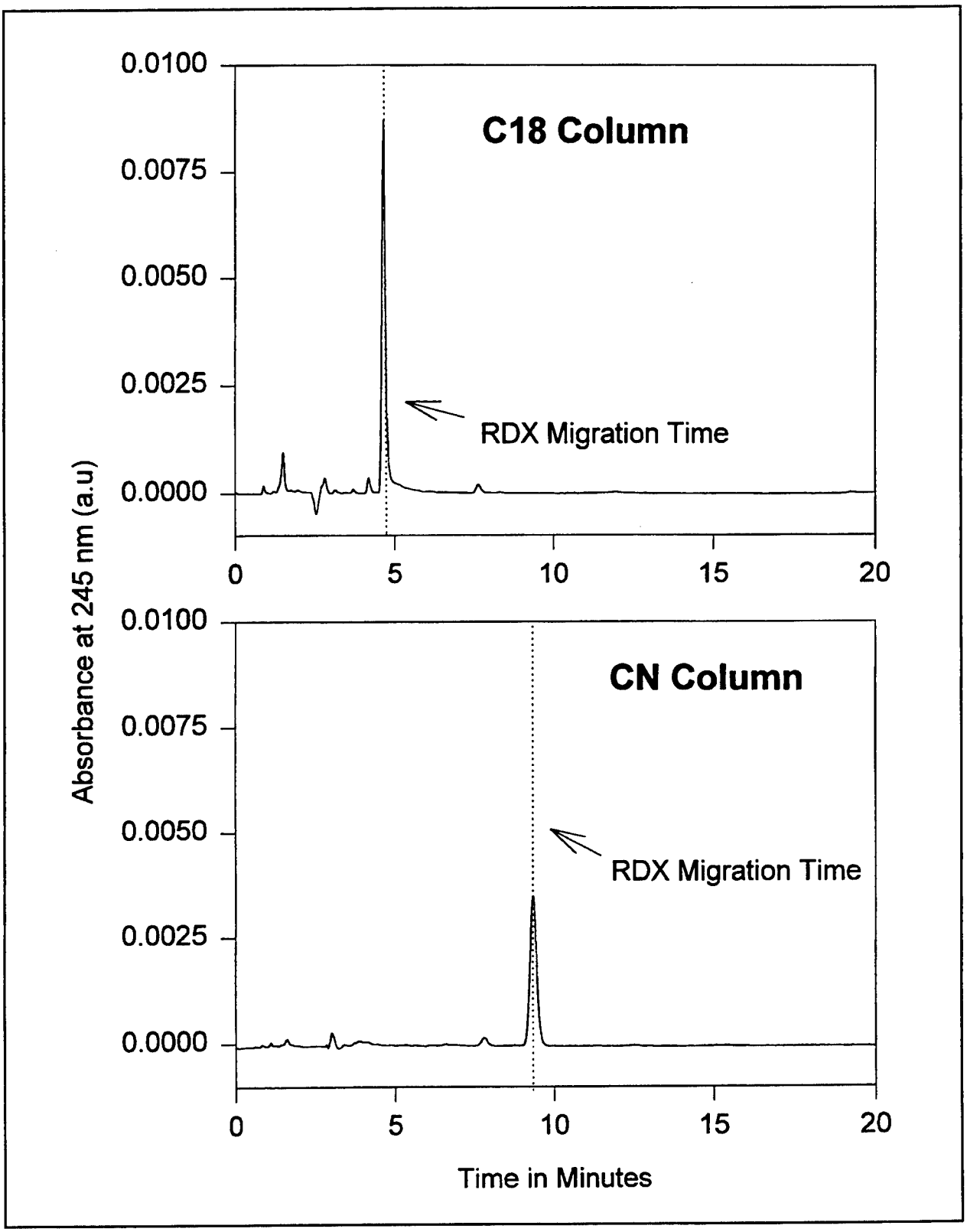

Figure 14. Chromatograms of RDX containing lettuce

Table 3
Summary of Qualitative and Quantitative Determinations of RDX in Plants by Tissue Type

Tissue Type	$\frac{C18[RDX]-CN[RDX]}{CN[RDX]}$	RDX on Cn Column (Qual/Quant)	RDX on C18 Column (Qual/Quant)
Corn silage	-0.00104	+/+	+/+
Cyperus	-0.03682	+/+	+/+
Lettuce	0.071106	+/+	+/?
Radish root	5.482143	+/+	+/-
Green tomato fruit	3.636364	+/+	+/?
Red tomato fruit	-0.10429	+/?	+/+
Tomato plant	-0.43832	+/?	-/-

number indicates the opposite (e.g., tomato plant), indicating a natural interference at the RDX retention time on the CN column, which is artificially increasing the peak height attributed to that analyte.

The remaining columns in Table 3 summarize the ability to qualitatively and quantitatively determine RDX on each column. A positive (+) signifies that extracts of this tissue type can be definitively qualified or quantified above the detection limits described earlier on the specified column. A questionable (?) signifies that extracts of this tissue type can be definitively qualified or quantified only when high concentrations are present on the specified column. A negative (-) signifies that extracts of this tissue type cannot be definitively qualified or quantified even when high concentrations are present on the specified column. Generally, a positive quantitation on one column and a positive qualification on the other column is satisfactory for analytical determination (i.e., at least at +/+ and +/-).

Figures 15 and 16 display chromatograms of extracts from corn tissues obtained using C18 and CN analytical columns. Corn was grown with irrigation water that contained approximately 5 ppm RDX. After the corn was harvested, it was dissected into four plant organ types: leaves, husk, stalk, and tassels. These samples were homogenized, freeze-dried, extracted, and analyzed according to the preceding method. As can be seen in Figures 15 and 16, only the leaves and the tassels contained significant amounts of RDX.

The ability to determine degradation products of explosives is a valuable tool for answering a number of questions regarding plant/contaminant interactions. Of large concern is the possibility that following contaminant uptake, natural metabolic action of the plants may transform environmentally damaging contaminants and produce compounds that have higher toxicity, greater bioavailability, or higher mobility in the environment than the original contaminant. Observing TNT degradation products like 4-A- and 2-A-DNT formation during phytoremediation allows researchers to propose possible degradation pathways and to better understand the transformations performed by plant metabolism.

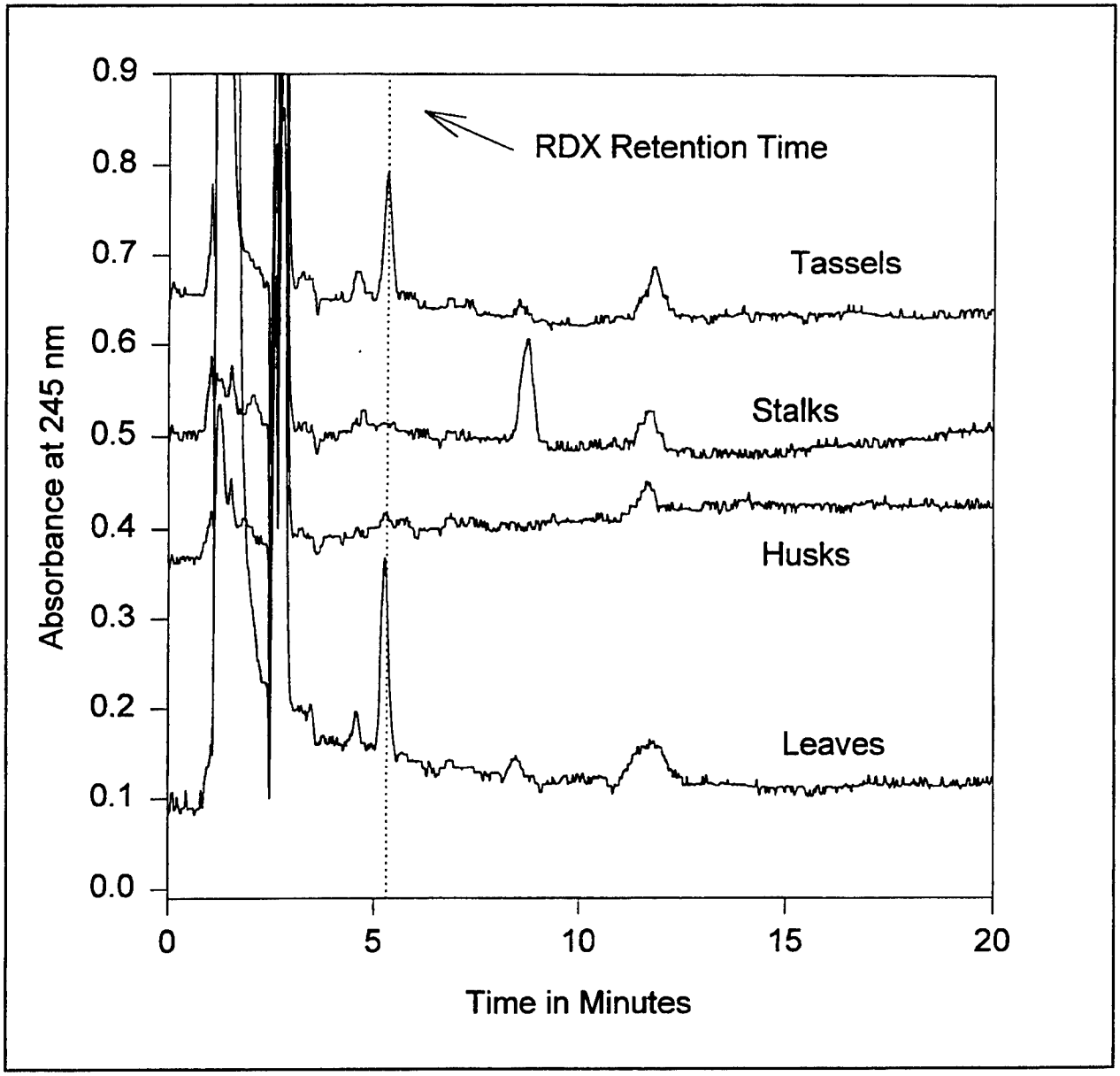

Figure 15. Corn plant organs on C18 column

Studies were performed in which the MDL and laboratory reporting limits (LRL) were determined for the explosives in a range of plant tissues. Samples were prepared from unexposed reference plant material as described in the Chapter 2. Table 4 contains the results of seven replicate runs near the data reporting limit as well as the statistical interpretation of those results. Through the sample preparation and cleanup process, a concentration factor was introduced that depended on the masses of the plant tissues tested, the amount of water removed during sample preparation, and the volume of solvents used for cleanup and mobile phase matching. The results are presented in concentration units that reflect the levels of the spiking components in the sample as it was injected onto

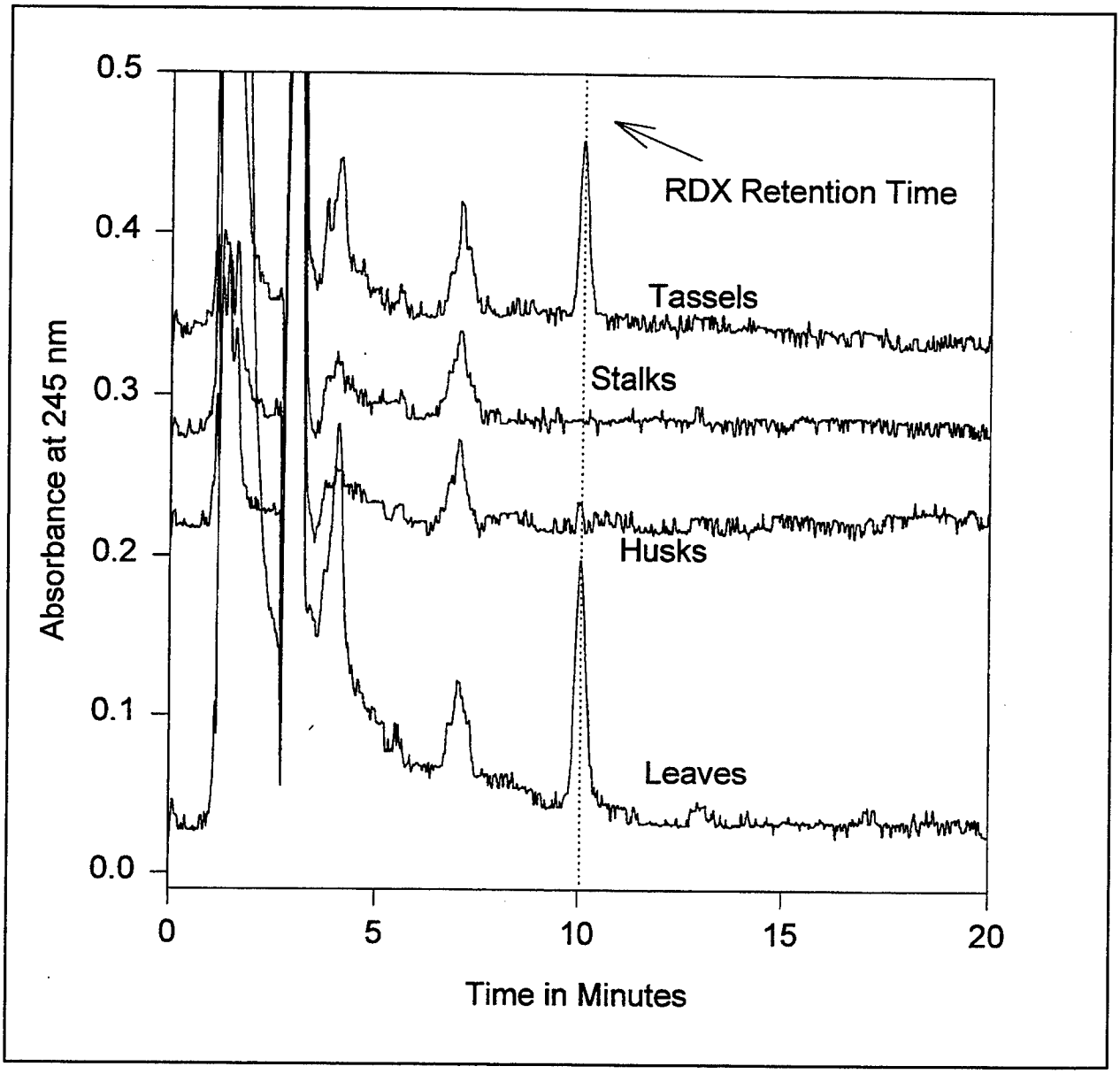

Figure 16. Corn plant organs on CN column

the column (concentration factors for the various species along with the fresh and dry weights are listed in Table 5). Good precision was obtained for the replicate analysis in both water and soil matrices. The EPA SW846 (EPA 1992) method for determination of MDL and LRL was used to achieve the results presented in Table 4. Table 6 provides a detailed description of method detection limits for RDX for various plant matrixes. As can be seen, large variations in detection limits are observed for different plant matrixes.

Table 4
MDL Results by Tissue Type-Spiking After Freeze-Drying

Compound		Conc mg/L	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	AVG mg/L	STD DEV	MDL mg/L	% Rec	LRL mg/L
Corn Fruit														
C18	HMX	0.125	0.099	0.113	0.127	0.094	0.096	0.088	0.090	0.101	0.014	0.042	80.80	0.141
CN		0.125	0.087	0.096	0.117	0.087	0.084	0.084	0.086	0.092	0.012	0.036	73.26	0.119
C18	RDX	0.125	0.116	0.115	0.122	0.119	0.102	0.113	0.118	0.115	0.006	0.019	92.00	0.064
CN		0.125	0.119	0.117	0.124	0.121	0.112	0.126	0.118	0.120	0.005	0.014	95.66	0.047
C18	TNB	0.125	0.118	0.119	0.123	0.119	0.108	0.118	0.119	0.118	0.005	0.014	94.17	0.046
CN														
C18	4-A-DNT													
CN		0.125	0.125	0.125	0.127	0.133	0.123	0.128	0.132	0.128	0.004	0.011	102.06	0.037
C18	24-DNT	0.125	0.125	0.122	0.127	0.123	0.121	0.121	0.111	0.121	0.005	0.015	97.14	0.051
CN		0.125	0.123	0.120	0.126	0.125	0.128	0.122	0.119	0.123	0.003	0.010	98.63	0.033
C18	TNT	0.125	0.120	0.119	0.124	0.117	0.107	0.114	0.118	0.117	0.005	0.016	93.60	0.054
CN		0.125	0.121	0.119	0.126	0.123	0.117	0.125	0.118	0.121	0.004	0.010	97.03	0.035
C18	26-DNT	0.125	0.087	0.085	0.086	0.082	0.090	0.087	0.085	0.086	0.002	0.007	68.80	0.024
CN		0.125	0.085	0.084	0.085	0.089	0.083	0.087	0.084	0.085	0.002	0.006	68.23	0.021
C18	NB	0.125	0.091	0.090	0.092	0.085	0.097	0.091	0.088	0.091	0.004	0.011	72.40	0.035
CN		0.125	0.094	0.091	0.092	0.089	0.096	0.091	0.093	0.092	0.002	0.007	73.83	0.023
C18	DNA	0.125	0.085	0.084	0.085	0.081	0.089	0.084	0.083	0.084	0.002	0.007	67.54	0.024
CN		0.125	0.088	0.085	0.086	0.086	0.009	0.009	0.087	0.064	0.038	0.114	51.36	0.379
C18	Tetryl	0.125	0.122	0.132	0.141	0.139	0.138	0.136	0.130	0.134	0.007	0.020	107.20	0.066
CN		0.125	0.133	0.129	0.113	0.119	0.142	0.141	0.135	0.130	0.011	0.033	104.23	0.109

(Sheet 1 of 5)

Table 4 (Continued)

Compound		Conc mg/L	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	AVG mg/L	STD DEV	MDL mg/L	% Rec	LRL mg/L
Corn Silage														
C18	HMX	0.125	0.122	0.123	0.126	0.121	0.134	0.126	0.122	0.125	0.004	0.013	99.89	0.045
CN		0.125	0.106	0.100	0.102	0.105	0.106	0.100	0.100	0.103	0.003	0.009	82.17	0.029
C18	RDX	0.125	0.113	0.113	0.117	0.119	0.113	0.114	0.113	0.115	0.002	0.007	91.66	0.024
CN		0.125	0.119	0.119	0.126	0.123	0.123	0.119	0.125	0.122	0.003	0.009	97.60	0.030
C18	TNB	0.125	0.112	0.113	0.120	0.123	0.115	0.116	0.112	0.116	0.004	0.013	92.69	0.042
CN														
C18	4-A-DNT													
CN		0.125	0.122	0.127	0.123	0.126	0.128	0.125	0.123	0.125	0.002	0.007	99.89	0.023
C18	24-DNT	0.125	0.114	0.114	0.121	0.115	0.122	0.116	0.114	0.117	0.003	0.010	93.26	0.035
CN		0.125	0.127	0.121	0.124	0.129	0.125	0.121	0.127	0.125	0.003	0.009	99.89	0.031
C18	TNT	0.125	0.110	0.111	0.118	0.108	0.111	0.114	0.114	0.112	0.003	0.010	89.83	0.033
CN		0.125	0.116	0.124	0.118	0.120	0.122	0.120	0.120	0.120	0.003	0.008	96.00	0.026
C18	26-DNT	0.125	0.085	0.083	0.101	0.085	0.084	0.095	0.084	0.088	0.007	0.021	70.51	0.070
CN		0.125	0.085	0.087	0.085	0.103	0.086	0.101	0.086	0.090	0.008	0.024	72.34	0.080
C18	NB	0.125	0.091	0.090	0.108	0.090	0.091	0.101	0.090	0.094	0.007	0.022	75.54	0.072
CN		0.125	0.091	0.093	0.092	0.111	0.093	0.110	0.096	0.098	0.009	0.026	78.40	0.087
C18	DNA	0.125	0.085	0.084	0.101	0.084	0.084	0.094	0.084	0.088	0.007	0.020	70.40	0.068
CN		0.125	0.085	0.087	0.105	0.087	0.088	0.103	0.088	0.092	0.008	0.025	73.49	0.084
C18	Tetryl	0.125	0.130	0.138	0.130	0.121	0.127	0.130	0.116	0.127	0.007	0.021	101.94	0.071
CN		0.125	0.122	0.130	0.126	0.125	0.124	0.119	0.133	0.126	0.005	0.014	100.46	0.047

(Sheet 2 of 5)

Table 4 (Continued)

Compound		Conc mg/L	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	AVG mg/L	STD DEV	MDL mg/L	% Rec	LRL mg/L
Cyperus esculentus														
C18	HMX	0.125	0.000	0.000	0.000	0.000	0.000	0.130	0.124	0.036	0.062	0.186	29.030	0.620
CN		0.125	0.000	0.000	0.000	0.000	0.000	0.114	0.132	0.035	0.060	0.181	28.11	0.602
C18	RDX	0.125	0.134	0.132	0.126	0.128	0.122	0.120	0.125	0.127	0.005	0.015	101.37	0.051
CN		0.125	0.127	0.125	0.125	0.125	0.117	0.121	0.125	0.124	0.003	0.010	98.86	0.034
C18	TNB	0.125	0.130	0.130	0.122	0.124	0.109	0.114	0.110	0.120	0.009	0.027	95.89	0.089
CN														
C18	4-A-DNT													
CN		0.125	0.134	0.129	0.127	0.130	0.124	0.123	0.136	0.129	0.005	0.014	103.20	0.048
C18	24-DNT	0.125	0.135	0.135	0.124	0.127	0.112	0.121	0.116	0.124	0.009	0.026	99.43	0.088
CN		0.125	0.134	0.131	0.127	0.127	0.122	0.121	0.130	0.127	0.005	0.014	101.94	0.047
C18	TNT	0.125	0.125	0.125	0.116	0.113	0.117	0.116	0.109	0.117	0.006	0.018	93.83	0.059
CN		0.125	0.126	0.126	0.121	0.115	0.117	0.116	0.123	0.121	0.005	0.014	96.46	0.047
C18	26-DNT	0.125	0.108	0.107	0.110	0.114	0.112	0.104	0.108	0.109	0.003	0.010	87.20	0.033
CN		0.125	0.104	0.108	0.108	0.109	0.107	0.107	0.105	0.107	0.002	0.005	85.49	0.018
C18	NB	0.125	0.107	0.108	0.113	0.113	0.111	0.106	0.109	0.110	0.003	0.008	87.66	0.028
CN		0.125	0.108	0.111	0.112	0.109	0.109	0.107	0.111	0.110	0.002	0.005	87.66	0.018
C18	DNA	0.125	0.105	0.107	0.111	0.110	0.109	0.105	0.107	0.108	0.002	0.007	86.17	0.024
CN		0.125	0.104	0.106	0.106	0.107	0.105	0.104	0.102	0.105	0.002	0.005	83.89	0.017
C18	Tetryl	0.125	0.067	0.033	0.038	0.040	0.044	0.045	0.045	0.045	0.011	0.032	35.66	0.108
CN		0.125	0.051	0.030	0.036	0.035	0.036	0.042	0.054	0.041	0.009	0.027	32.46	0.089

(Sheet 3 of 5)

Table 4 (Continued)

Compound		Conc mg/L	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	AVG mg/L	STD DEV	MDL mg/L	% Rec	LRL mg/L
							Lettuce							
C18	HMX	0.125	0.085	0.081	0.073	0.086	0.081	0.077	0.067	0.079	0.007	0.020	62.86	0.068
CN		0.125	0.079	0.062	0.066	0.061	0.063	0.064	0.057	0.065	0.007	0.021	51.66	0.069
C18	RDX		0.112	0.118	0.113	0.118	0.119	0.118	0.111	0.116	0.003	0.010	92.46	0.034
CN		0.125	0.126	0.122	0.124	0.120	0.122	0.123	0.118	0.122	0.003	0.008	97.71	0.026
C18	TNB	0.125	0.109	0.117	0.118	0.115	0.117	0.118	0.111	0.115	0.004	0.011	92.00	0.036
CN														
C18	4-A-DNT													
CN		0.125	0.122	0.124	0.126	0.125	0.126	0.126	0.123	0.125	0.002	0.005	99.66	0.016
C18	24-DNT	0.125	0.117	0.120	0.122	0.119	0.121	0.120	0.116	0.119	0.002	0.006	95.43	0.021
CN		0.125	0.125	0.126	0.130	0.126	0.124	0.131	0.122	0.126	0.003	0.010	101.03	0.032
C18	TNT	0.125	0.110	0.117	0.106	0.114	0.362	0.368	0.414	0.227	0.145	0.435	181.83	1.451
CN		0.125	0.114	0.119	0.110	0.117	0.352	0.351	0.393	0.222	0.135	0.403	177.83	1.346
C18	26-DNT	0.125	0.087	0.090	0.097	0.103	0.091	0.093	0.094	0.094	0.005	0.016	74.86	0.052
CN		0.125	0.090	0.090	0.092	0.089	0.090	0.088	0.092	0.090	0.001	0.004	72.11	0.015
C18	NB	0.125	0.091	0.096	0.103	0.091	0.094	0.090	0.097	0.095	0.005	0.014	75.66	0.046
CN		0.125	0.094	0.095	0.100	0.092	0.092	0.094	0.094	0.094	0.003	0.008	75.54	0.027
C18	DNA	0.125	0.089	0.092	0.100	0.090	0.093	0.088	0.087	0.091	0.004	0.013	73.03	0.044
CN		0.125	0.096	0.090	0.089	0.088	0.088	0.089	0.087	0.090	0.003	0.009	71.66	0.030
C18	Tetryl	0.125	0.062	0.046	0.043	0.043	0.047	0.051	0.053	0.049	0.007	0.020	39.43	0.068
CN		0.125	0.040	0.067	0.057	0.042	0.060	0.060	0.063	0.056	0.010	0.031	44.46	0.104

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Table 4 (Concluded)

Compound		Conc mg/L	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	AVG mg/L	STD DEV	MDL mg/L	% Rec	LRL mg/L
Tomato														
C18	HMX	0.125	0.100	0.097	0.099	0.099	0.102	0.112	0.099	0.101	0.005	0.015	80.91	0.050
CN		0.125	0.090	0.089	0.093	0.094	0.095	0.104	0.092	0.094	0.005	0.015	75.09	0.049
C18	RDX	0.125	0.117	0.114	0.113	0.113	0.117	0.116	0.116	0.115	0.002	0.005	92.11	0.018
CN		0.125	0.125	0.123	0.120	0.125	0.125	0.125	0.123	0.124	0.002	0.006	98.97	0.019
C18	TNB	0.125	0.117	0.117	0.117	0.117	0.121	0.117	0.117	0.118	0.002	0.005	94.06	0.015
CN														
C18	4-A-DNT													
CN		0.125	0.119	0.116	0.118	0.116	0.116	0.118	0.118	0.117	0.001	0.004	93.83	0.013
C18	TNT	0.125	0.124	0.122	0.117	0.117	0.125	0.120	0.120	0.121	0.003	0.009	96.57	0.031
CN		0.125	0.117	0.116	0.114	0.116	0.121	0.119	0.117	0.117	0.002	0.007	93.71	0.023
C18	24-DNT	0.125	0.123	0.121	0.123	0.121	0.126	0.123	0.122	0.123	0.002	0.005	98.17	0.017
CN		0.125	0.120	0.118	0.118	0.118	0.120	0.120	0.118	0.119	0.001	0.003	95.09	0.011
C18	26-DNT	0.125	0.099	0.101	0.102	0.099	0.103	0.102	0.102	0.101	0.002	0.005	80.91	0.016
CN		0.125	0.103	0.101	0.102	0.100	0.102	0.100	0.102	0.101	0.001	0.003	81.14	0.011
C18	NB	0.125	0.101	0.103	0.101	0.104	0.104	0.101	0.107	0.103	0.002	0.006	82.34	0.021
CN		0.125	0.107	0.107	0.103	0.105	0.104	0.103	0.107	0.105	0.002	0.006	84.11	0.019
C18	DNA	0.125	0.096	0.098	0.096	0.099	0.098	0.096	0.101	0.098	0.002	0.006	78.17	0.019
CN		0.125	0.103	0.101	0.099	0.102	0.102	0.101	0.104	0.102	0.002	0.005	81.37	0.016
C18	Tetryl	0.125	0.115	0.148	0.125	0.150	0.117	0.176	0.161	0.142	0.023	0.070	113.37	0.233
CN		0.125	0.137	0.176	0.156	0.175	0.177	0.173	0.127	0.160	0.021	0.062	128.11	0.207

(Sheet 5 of 5)

Table 5
Fresh and Dry Weight Concentration Factors by Tissue Type

Plant Species	Fresh Weight (g)/ Concentration Factor (mL acn/kg fresh)	Dry Weight (g)/ Concentration Factor (mL acn/kg dry)
Corn silage	102.3/15.2	19.4/80
Corn kernels	89.9/26.3	29.6/80
Cyperus	104.2/14.4	18.7/80
Lettuce	99.8/3.5	4.39/80
Tomato	97.6/5.4	6.70/80

Table 6
Seven Replicates Spiked at 0.125 ppm Prior to Extraction MDL for RDX (ppm)

	Cyperus	Silage	Corn	Lettuce	Tomato
Average of seven replicate analyses	0.127	0.112	0.120	0.122	0.124
MDL-injected	0.003	0.009	0.014	0.003	0.005
MDL-fresh weight	0.049 (79.5% water)	0.165 (77.1% water)	0.380 (66.1% water)	0.012 (95.17% water)	0.027 (93.1% water)
MDL-dry weight	0.24	0.72	1.12	0.24	0.40

Concentration Ranges

The tested concentration range is dependent on the matrix in which the explosives, by-products of explosives manufacture, and explosives degradation products are being measured. Standards spiked into homogenized corn samples can be detected in the concentration range between 0.01 and 5 mg/L in the injected extract solution, which corresponds to 0.08 to 40 mg/kg fresh weight (assuming 90-percent water in plant tissue) and 0.8 to 400 mg/kg dry weight. The testable concentration range will vary considerably with the matrix encountered. Generally, the cleaner the sample, the less background current is detected at the detection wavelength providing lower detection limits. Samples that are highly contaminated with other contaminants may have much higher backgrounds associated with them, and detection limits are considerably higher.

The concentration range that can be accurately tested by this method is bounded by two extremes because of the nature of the ultraviolet detection system. In the first, the concentration of the analyte is so low that it does not produce an

absorbance at the monitored wavelength greater than the noise of the background absorbance. In the second, the concentration is so large that the absorbance due to that component is no longer linear with respect to concentration.

In a flow-through cell, the actual amount of absorbance detected depends on the rate of flow through the cell. At a high flow rate, the component absorbs the radiation at the monitoring wavelength for a short period of time. At slower flow rates, the analyte spends more time in the detection cell, and more energy is absorbed by the compounds being detected and by interferences that absorb at 245 nm. In the work described here, the flow rate is 1.2 mL/min. Increased sensitivity can often be achieved at lower flow rates; however, lower flow rates increase analysis time.

Purified reference standards of the analytes were used to prepare solutions with concentrations of 0.05, 0.1, 0.4, 1.0, and 4.0 mg/L (which corresponds to 0.4, 1.6, 4, 12, and 40 mg/kg fresh weight (assuming 90-percent water) or 4, 16, 40, 120, and 400 mg/kg dry weight) for instrument calibration. Stock sources were prepared from neat or crystalline stock explosives standards obtained from the Army Environmental Center at Aberdeen Proving Ground. Excellent linearity is achieved over two orders of magnitude of concentration range. This allows for quantitation of the explosive compounds. Retention times are stable throughout the two orders of magnitude in the calibrated concentration range.

Comparison of Modified Method with EPA SW-846 Method 8330 for Soils and Sediments

The modifications to Method 8330 for soil that make the analysis of plant tissues possible are a result of both the unique matrix conditions encountered when analyzing plant tissues and the characteristics of the analytes. Table 7 provides a summary of the procedural steps involved in Method 8330 for soils as well as the modifications made for plant tissues. The modifications are such that a laboratory that currently performs Method 8330 for soils and sediments will be able to perform the modified method for plant tissues without a significant increase in costs. The only equipment required that is not utilized for Method 8330 for soils and sediments is the laboratory mill and freeze-dryer. These common laboratory items are present in many laboratories. The approximate time required for analysis of plants compared with the time required for analysis of soils using the two methods is summarized in Table 8 below. The approximate total time for method completion for the two methods are comparable. The method modified for plant tissues takes slightly longer than the soils method due to the extended time involved in homogenization, freezing, freeze-drying, and analysis. The time involved in data analysis, calculation, and reporting is expected to be similar for the two methods.

Table 7
Comparison of Method Process Steps

	Process Step	8330-For Soil	8330 Modified for Plant Tissues
1	Collection/storage	Refrigerated.	Frozen.
2	Air-drying	Dry to constant weight, avoiding sunlight.	Not used.
3	Homogenization	Grind with mortar and pestle to pass a 30 mesh screen.	Liquefy with cooled high-speed laboratory mill.
4	Freeze-drying	Not used.	Freeze-dry frozen homogenate to constant weight.
5	Extraction	18 hr, cooled sonic bath.	18 hr, cooled sonic bath.
6	Cleanup	Not used.	Silica/florisil chromatographic cleanup.
7	Analysis	HPLC, CN, and C18 columns run time = 20 min.	HPLC, CN, and C18 columns run time = 20-45 min.

Table 8
Comparison of Time Required for Method Process Steps

	Process Step	8330—For Soil (Approximate time per sample in minutes)	8330 Modified for Plant Tissues (Approximate time per sample in minutes)
1	Collection/storage	---	---
2	Air-Drying	1,080 - 4,320	----
3	Homogenization	5 - 15	10 - 60
4	Freezing/freeze-drying	---	1,080 - 4,320
6	Extraction	1,080	1,080
7	Cleanup	---	30
8	Analysis[1]	20	20 - 45
	Total	2,185 - 5,435	2,220 - 5,535

[1] Two HPLC instruments, simultaneous analysis on CN and C18 columns.

Table 9 displays a comparison of three method performance parameters for the two methods. Estimated reporting limits for the two methods are presented. The values presented for Method 8330 for soils and sediments are found in EPA SW-846 Method 8330 (EPA 1992); the values for the modified method for plant tissues are the LRL, which is three times the calculated MDL. The estimated reporting limits for soils are given as dry weight because water usually makes up a small percentage of sample mass for these matrixes, and the difference between dry and wet weight values is small. Plant tissues, however, are mostly water, and

Table 9
Comparison of Method Performance Factors

Method Performance Factor	Analyte	8330-For Soil (Soil spiked prior to extraction) ppm	8330 Modified for Plant Tissues (Tomato fruit spiked prior to extraction), ppm	
			Dry Wt.	Fresh Wt.
Estimated Reporting Limits	HMX	2.20	4.01	0.64
	RDX	1.00	1.51	0.24
	Tetryl	0.65	18.65	2.96
	1,3,5-TNB	0.25	1.21	0.19
	NB	0.25	1.67	0.27
	TNT	0.25	2.80	0.27
	2- OR 4-A-DNT	---	1.00	0.16
	2,4-DNT	0.25	1.36	0.22
	2,6-DNT	0.26	1.26	0.20
Single Laboratory Precision (% RSD)	HMX	3.70	4.95	
	RDX	2.30	1.53	
	TNB	4.60	1.28	
	TNT	3.50	2.61	
	2,4-DNT	4.70	1.39	
	Tetryl	17.90	16.3	
% Recovery	HMX	95.0	80.9	
	RDX	96.0	99.0	
	TNB	89.0	94.0	
	TNT	98.0	96.6	
	2,4-DNT	96.0	98.2	
	Tetryl	58.0	113.4	

the dry and fresh weight reporting levels are significantly different. For this reason, both the dry and fresh weight reporting levels are presented. As can be seen, the levels at which explosive analytes can be reported in fresh plant tissues is comparable with those for soils. When reported as dry weight, the reporting limits are 5 to 10 times higher for plant tissues compared with soils. The other method performance parameters shown are single laboratory precision and percent recovery for preextraction spikes (soil values taken from EPA SW-846 Method 8330 (EPA 1992)). Comparison of the results of both of these factors shows similar method performance of the modified method for plant tissues and Method 8330 for soils.

4 Conclusions

Application of EPA Method 8330 for analysis of explosives in soils could not be used as is for the analysis of explosives in plants. A means of separation and quantitation of explosives in plant tissues has been developed and shown to produce results that are statistically significant and viable. Laboratories equipped for analysis of explosives by Method 8330 can perform the analysis of plant tissues without additional equipment other than a laboratory mill and freeze-dryer. The analysis time for the modified method is similar to that for Method 8330 for soils. The method has been used for determining the extent of bioaccumulation of RDX in garden vegetables. Aquatic plant tissues have been analyzed, and the results of these analyses have provided a basis for screening plants that are capable of degrading explosives. Plant uptake of explosives from finished soils produced by composting and bioslurry reactors has been studied using the modified method. This method has proven to be effective on a large range of matrices, is cost-effective, is easily implemented, and provides valuable information regarding risk assessment and remediative action.

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13. ABSTRACT (*Maximum 200 words*)

Knowledge of the concentration of the contaminants and the molecular state of their degradation products is helpful in assessing the environmental risks associated with the contaminants as well as evaluating the design of remediation technologies. A great deal of interest has been generated recently in the determination of explosives and explosives' by-products in plant tissues. The methods traditionally used for the analysis of explosives in solid matrixes are not adequate due to the high organic content of the plant tissues. Data that are obtained using analytical methods not designed for the specific matrix encountered may lead to incorrect quantitation of the target analyte. The methods used to detect the explosives and their degradation products require matrix-specific sample preparation, separation by reversed phase high performance liquid chromatography, and ultraviolet detection.

This report addresses three important points in connection with the analysis of explosives in plant tissues: extraction, interferences, and method performance. The extraction of the contaminants from the matrix requires a different set of extraction techniques from those used for standard water and soil extractions. These exotic matrixes contain much higher organic content than soil or water and, as a result, are prone to interference from biological molecules. A liquid chromatographic cleanup step is utilized to reduce these interferences. The performance of the modified method is reported with respect to method detection limits, analyte percent recoveries, and the methods applicability for analysis of contaminated plant tissues from plant uptake studies.

14. SUBJECT TERMS

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Bioavailability	Phytoremediation	Tissues
Explosives	Plant uptake	TNT

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